

77 Rec'd PCT/PTO 29 OCT 2001

TRANSMITTAL LETTER TO THE UNITED STATES

DESIGNATED/ELECTED OFFICE (DO/EO/US)

CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/926413

INTERNATIONAL APPLICATION NO.

PCT/FR00/01102

INTERNATIONAL FILING DATE

26 April 2000

PRIORITY DATE CLAIMED

29 April 1999

TITLE OF INVENTION

AMPHIPHILIC CYCLODEXTRINS, PREPARATION AND USE OF SAID CYCLODEXTRINS TO SOLUBILISE ORGANISED SYSTEMS AND INCORPORATE HYDROPHOBIC MOLECULES

APPLICANT(S) FOR DO/EO/US

Rachel AUZELY-VELTY, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☒ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:



13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PC/IB/304 Drawings (3 Sheets)

PCT/IB/308 Amended Sheets (Pages 16, 19 and 20)

Notice of Priority

Request for Consideration of Documents Cited in the International Search Report

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.101(b)(1))		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/926413		PCT/FR00/01102		215158US0PCT	
24. The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS PTO USE ONLY	
				\$890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20 - 20 =	0	x \$18.00	\$0.00	
Independent claims	5 - 3 =	2	x \$84.00	\$168.00	
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$1,058.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$0.00	
SUBTOTAL =				\$1,058.00	
Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
TOTAL NATIONAL FEE =				\$1,058.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL FEES ENCLOSED =				\$1,058.00	
				Amount to be refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of <u>\$1,058.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>15-0030</u> A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
<div style="display: flex; justify-content: space-between;"> <div> Telephone: (703)413-3000 Fax: (703)413-2220 </div> <div style="text-align: center;"> Surinder Sachar Registration No. 34,423 </div> </div>					
					
22850					
 SIGNATURE Norman F. Oblon NAME 24,618 REGISTRATION NUMBER <u>Oct. 29 2001</u> DATE					

215158US-0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

RACHEL AUZELY-VELTY ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: NEW U.S. PCT APPLN :
(BASED ON PCT/FR00/01102)

FILED: HEREWITH:

FOR: AMPHIPHILIC CYCLODEXTRINS,
PREPARATION AND USE OF SAID
CYCLODEXTRINS TO SOLUBILISE
ORGANISED SYSTEMS AND
INCORPORATE HYDROPHOBIC
MOLECULES

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

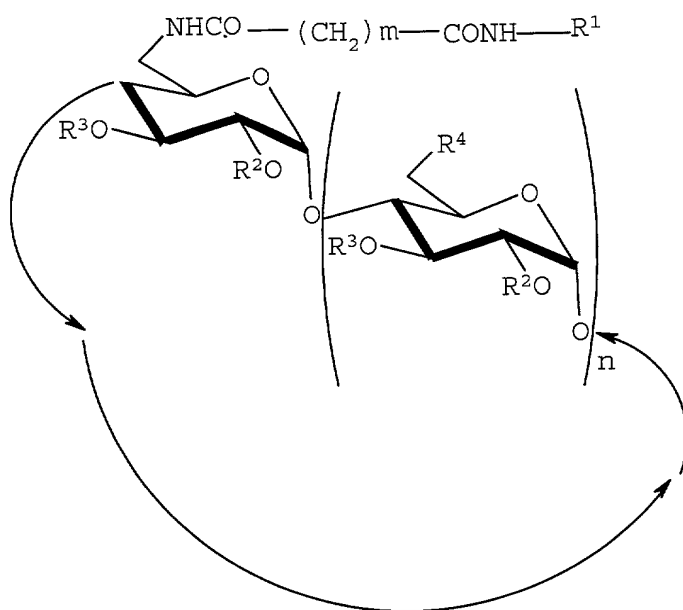
Prior to examination on the merits, please amend the above-identified application as follows.

IN THE CLAIMS

Please cancel Claims 1-16.

Please add the following new claims.

17. (New) Amphiphilic cyclodextrin derivative complying with the formula:



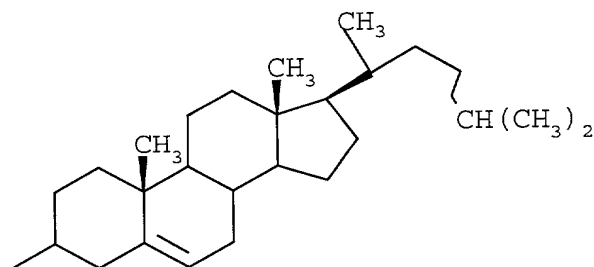
(I)

wherein:

- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H or R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and
- n is equal to 5, 6 or 7.

[illegible]

(III)

19. (New) Cyclodextrin derivative according to claim 17, wherein all the R^4 represent OR^2 .

20. (New) Cyclodextrin derivative according to claim 17, wherein R² represents the methyl group and R³ represents a hydrogen atom.

21. (New) Cyclodextrin derivative according to claim 17, wherein n is equal to 6.

22. (New) Cyclodextrin derivative according to claim 17, wherein m is equal to 2.

23. (New) Cyclodextrin derivative according to claim 18, wherein all the R⁴ represent OR².

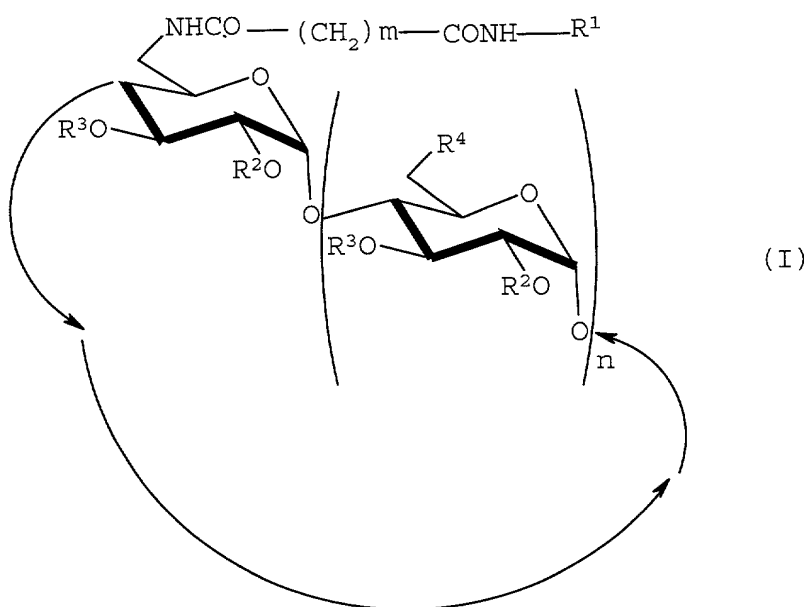
24. (New) Cyclodextrin derivative according to claim 18, wherein R² represents the methyl group and R³ represents a hydrogen atom.

25. (New) Cyclodextrin derivative according to claim 18, wherein n is equal to 6.

26. (New) Cyclodextrin derivative according to claim 18, wherein m is equal to 2.

27. (New) Mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',6',6'',6''', 6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose.

28. (New) Method to prepare a cyclodextrin derivative according to the formula:



wherein:

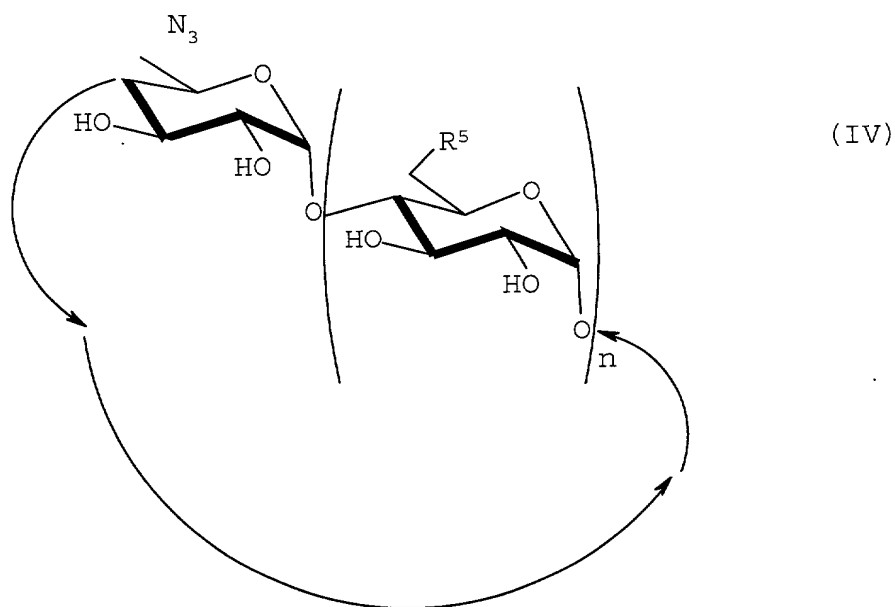
- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and
- n is equal to 5, 6 or 7,

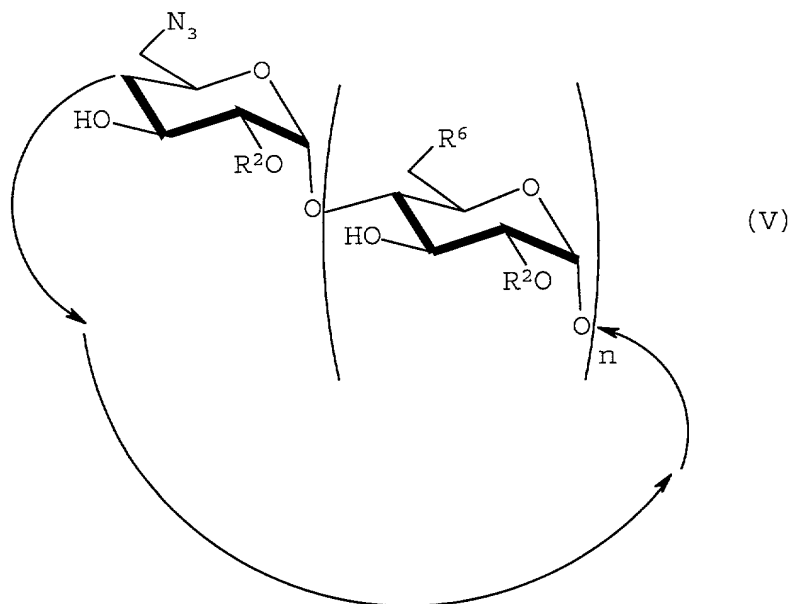
which comprises the following steps:

a) react a derivative according to the formula:



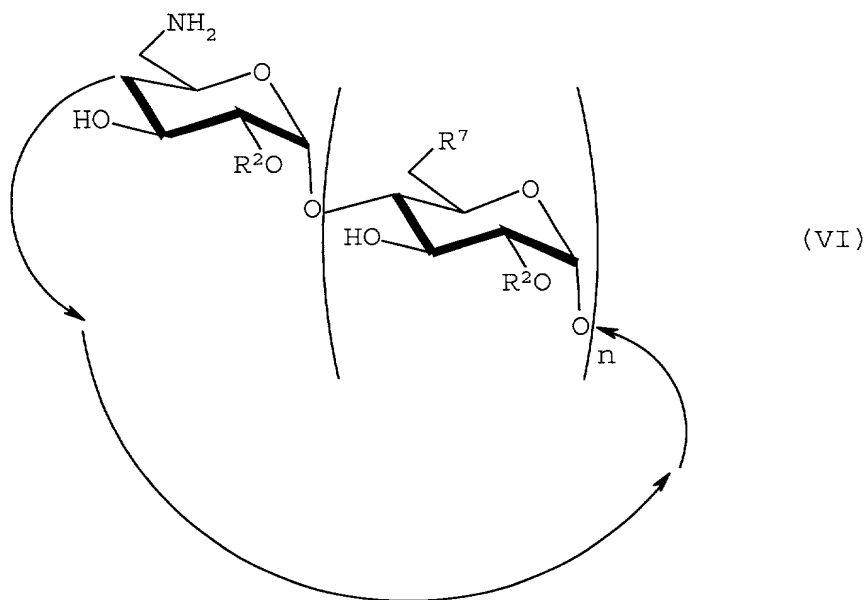
wherein all the R^5 represent OH, or one of the R^5 represents $-N_3$ and the other R^5 represent OH, provided that there is at least one glucose unit where R^5 represents OH between the two glucose units comprising the N_3 substituent, and n is equal to 5, 6 or 7,

with a dialkyl sulfate $SO_4R^2_2$ where R^2 has the significance given above, in a basic medium to obtain the cyclodextrin derivative according to the formula:



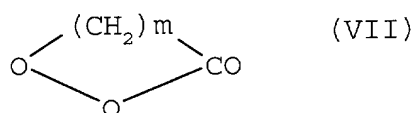
wherein all the R^6 represent OR^2 , or one of the R^6 represents N_3 and the other R^6 represent OR^2 , and R^2 and n are as defined above,

b) perform a Staudinger reaction on the derivative according to formula (V) using triphenylphosphine and ammonia to convert N_3 into NH_2 and obtain the derivative according to the formula:

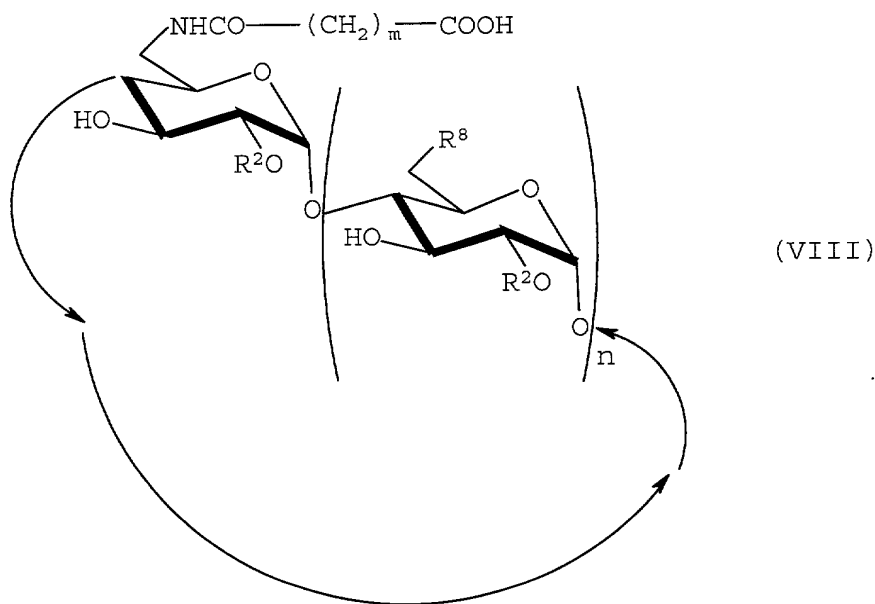


wherein all the R^7 represent OR^2 , or one of the R^7 represents NH_2 and the other R^7 represent OR^2 , and R^2 and n are as defined above,

c) react the derivative according to formula (VI) with an acid anhydride according to the formula:



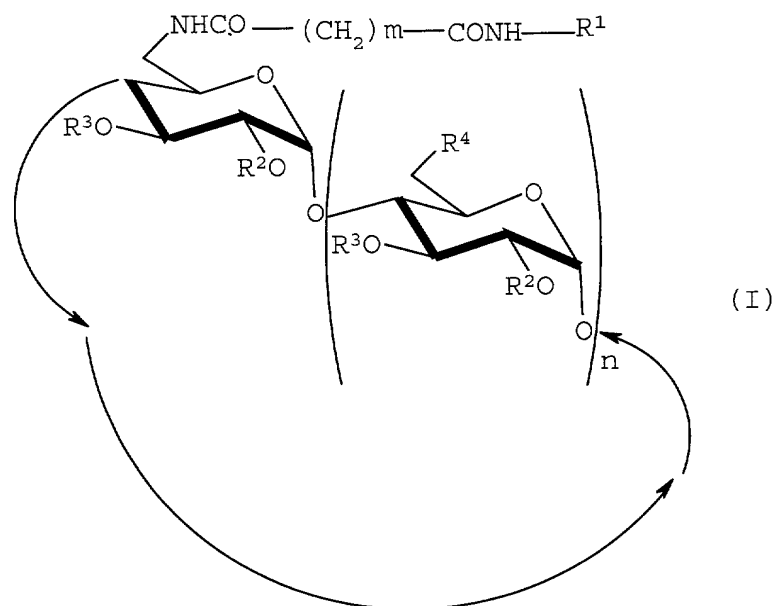
where m is as defined above, to obtain the derivative according to the formula:



wherein all the R^8 represent OR^2 , or one of the R^8 represents $—NHCO—(CH_2)_m—COOH$ and the other R^8 represent OR^2 , and R^2 , m and n are as defined above, and

d) react the derivative according to formula (VIII) with a compound according to the formula NH_2-R^1 to obtain the cyclodextrin derivative according to formula (I) defined above.

29. (New) Method to prepare a cyclodextrin derivative according to the following formula:



wherein:

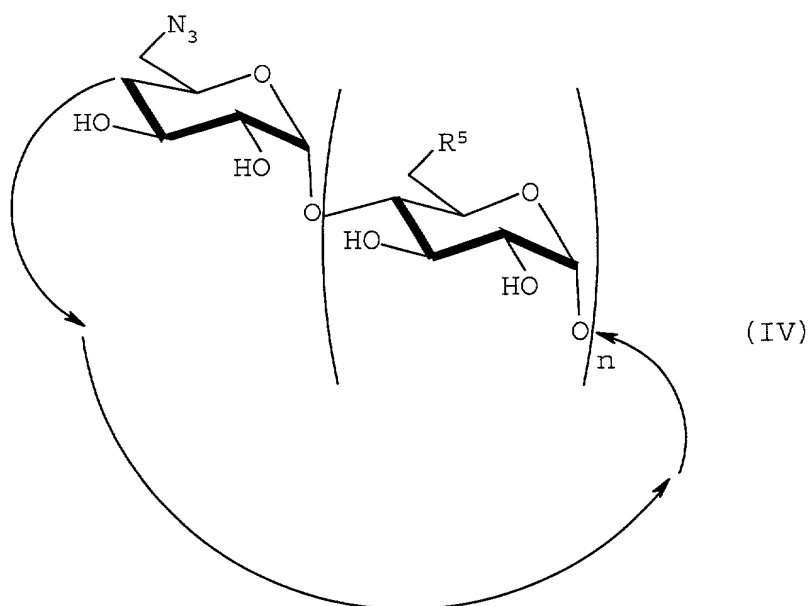
- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and
- n is equal to 5, 6 or 7,

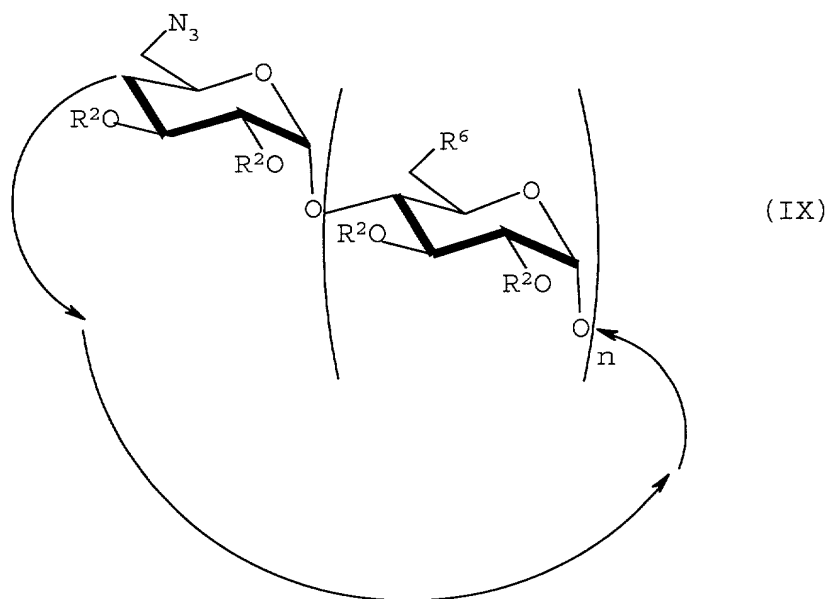
which comprises the following steps:

- a) react a derivative according to the formula:



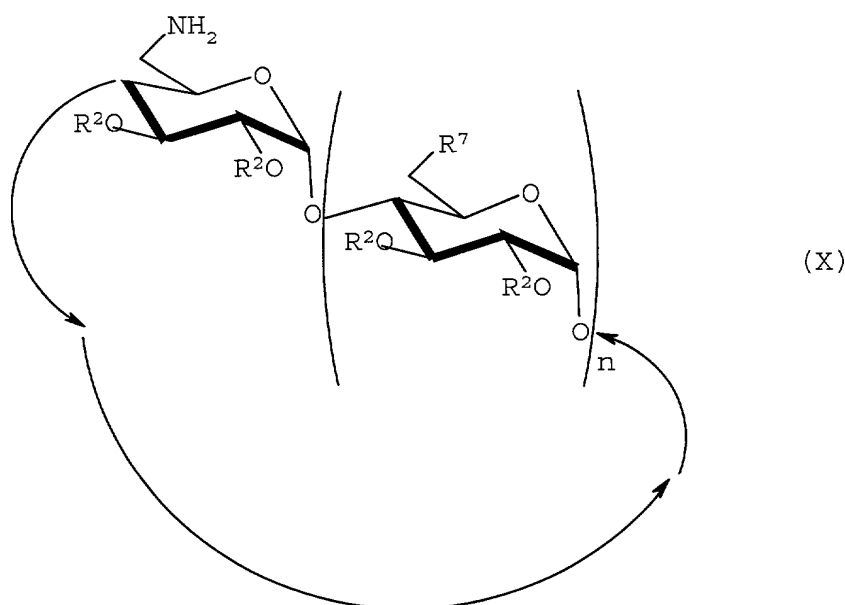
wherein all the R^5 represent OH, or one of the R^5 represents $-N_3$ and the other R^5 represent OH, provided that there is at least one glucose unit where R^5 represents OH between the two glucose units comprising the N_3 substituent, and n is equal to 5, 6 or 7,

with an iodoalkane according to the formula IR^2 wherein R^2 has the significance given above, in the presence of NaH to obtain the cyclodextrin derivative according to the formula:



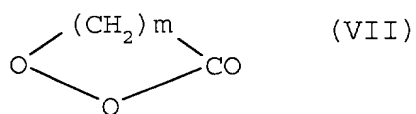
wherein all the R^6 represent OR^2 , or one of the R^6 represents N_3 and the other R^6 represent OR^2 , and R^2 and n are as defined above,

b) perform a Staudinger reaction on the derivative according to formula (IX) using triphenylphosphine and ammonia to convert N_3 into NH_2 and obtain the derivative according to the formula:

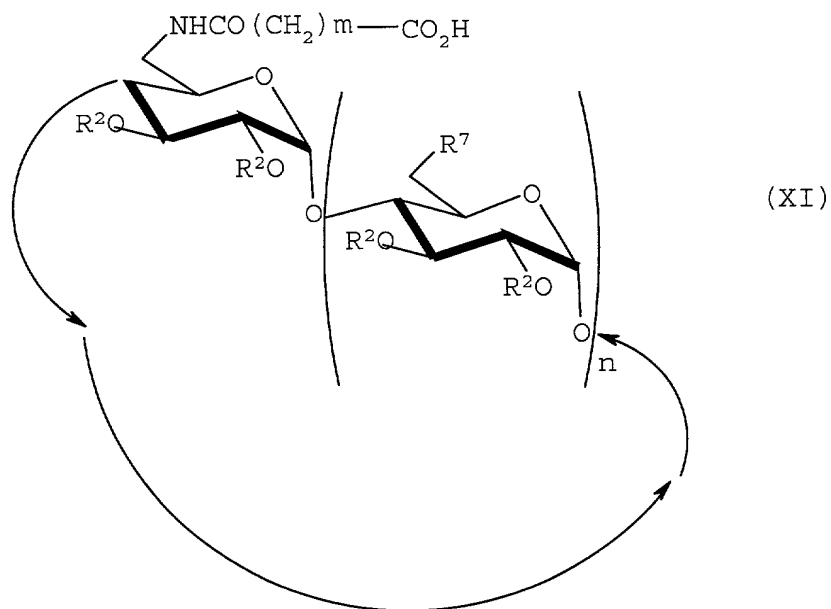


wherein all the R^7 represent OR^2 , or one of the R^7 represents NH_2 and the other R^7 represent OR^2 , and R^2 and n are as defined above,

c) react the derivative according to formula (X) with an acid anhydride according to the formula:



where m is as defined above, to obtain the derivative according to the formula:

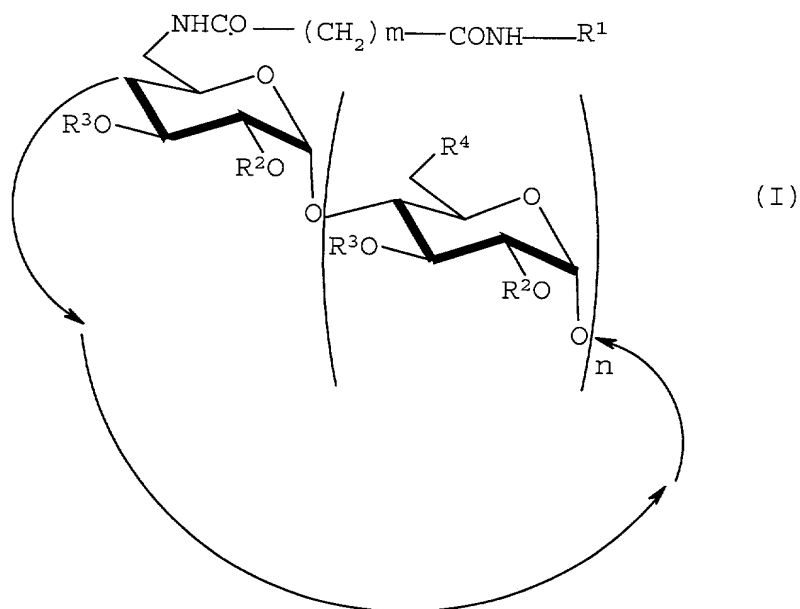


wherein all the R^7 represent OR^2 , or one of the R^7 represents $—NHCO—(CH_2)_m—COOH$ and the other R^7 represent OR^2 , and R^2 , m and n are as defined above, and

d) react the derivative according to formula (XI) with a compound according to the formula NH_2-R^1 to obtain the cyclodextrin derivative according to formula (I) defined above.

30. (New) Inclusion complex of a cyclodextrin derivative according to the following formula:

wherein:



- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H or R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

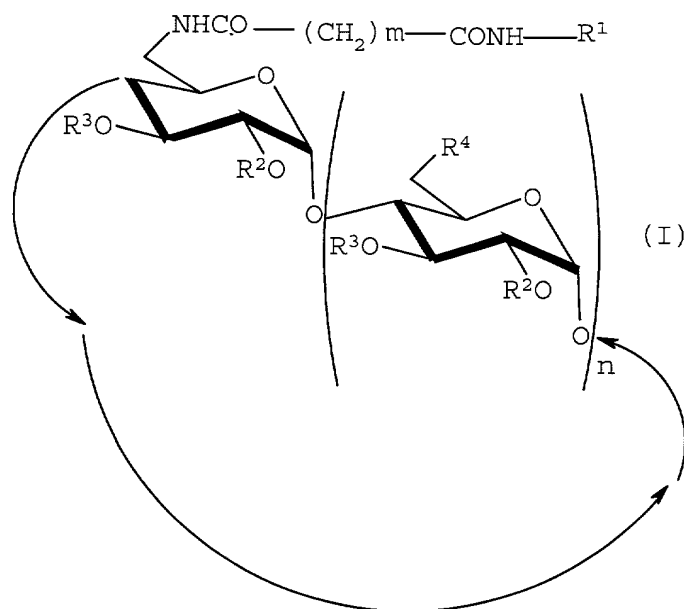
- m is an integer ranging from 1 to 8, and
- n is equal to 5, 6 or 7,

and a hydrophobic compound.

31. (New) Complex according to claim 30, wherein the hydrophobic compound is chosen from steroids, neurotropes, bacteriostatics, vitamins, vascular wall tonics and contrast agents.

32. (New) Complex according to claim 30, wherein the hydrophobic compound is chosen from 16-iodo-3-methylhexadecanoic acid, dothiepin, chloramphenicol, vitamin A and esculin.

33. (New) Aqueous solution of nanoparticles of a cyclodextrin derivative complying with the formula:



wherein:

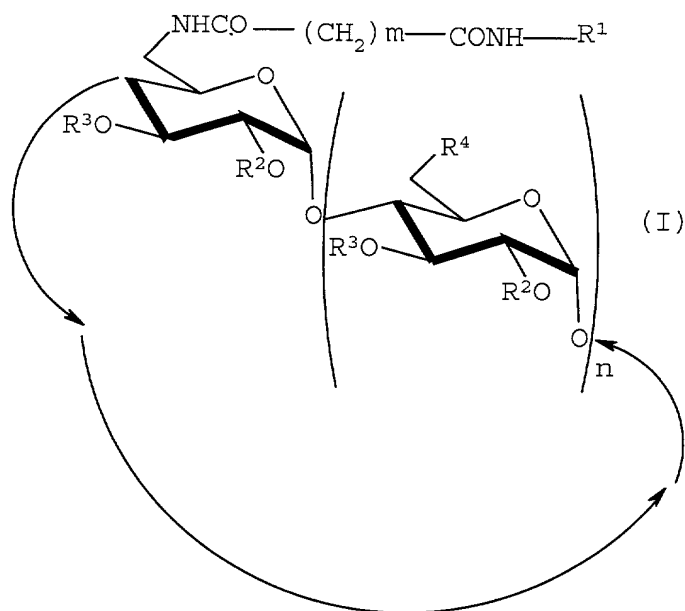
- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H or R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and
- n is equal to 5, 6 or 7,

or an inclusion complex according to claim 30.

34. (New) Organized surfactant system comprising a cyclodextrin derivative complying with the formula:



wherein:

- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H or R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

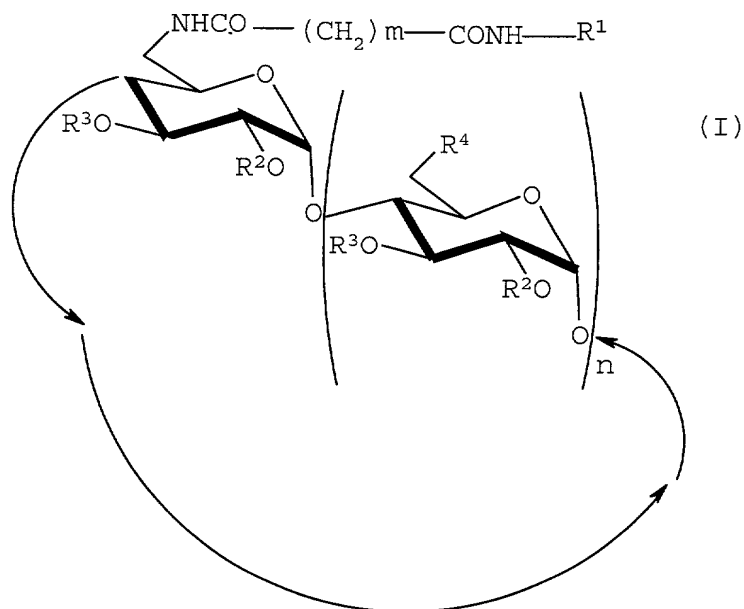
provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and
- n is equal to 5, 6 or 7,

or an inclusion complex according to claim 30.

35. (New) System according to claim 34 wherein the surfactant is a phospholipid.

36. (New) Aqueous solution comprising in solution a combined system formed from phospholipid or membrane protein vesicles, and at least one cyclodextrin derivative complying with the formula:



wherein:

- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H or R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2

provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and

- n is equal to 5, 6 or 7,

or at least one inclusion complex according to claim 30.

REMARKS

Claims 1-16 have been canceled. Claims 17-36 are new claims. Support for the new claims is found in the original claims. No new matter is added. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
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Registration No. 24,618

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Marked-Up Copy	
S e r i a l	N o :
Amendment Filed on:	
10-29-01	

IN THE CLAIMS

Claims 17-36 (New).

09/926413

3/pals 1

AMPHIPHILIC CYCLODEXTRINS, PREPARATION AND USE OF SAID
CYCLODEXTRINS TO SOLUBILISE ORGANISED SYSTEMS AND
INCORPORATE HYDROPHOBIC MOLECULES

Field of the invention

The present invention relates to new cyclodextrin derivatives, that can particularly be used to incorporate in aqueous media hydrophobic chemical compounds such as pharmaceutically active molecules,
5 molecules with cosmetic applications and molecules used as contrast agents for medical imaging.

More specifically, it relates to amphiphilic cyclodextrin derivatives showing self-organisation properties in aqueous media and liable to be
10 incorporated in organised surfactant systems resulting in the formation of combined systems.

Said incorporation in organised surfactant systems such as small phospholipid vesicles is intended to enable the transport of hydrophobic molecules included
15 in the cyclodextrin, for example an active ingredient, particularly by the transmembrane route, for example the transdermal route.

State of the related art

20 Cyclodextrins or cyclomaltooligosaccharides are natural compounds formed by the sequencing of 6, 7 or 8 glucose units bonded in α -1 \rightarrow 4 mode. Numerous studies have shown that these compounds could form inclusion complexes with hydrophobic molecules, thus enabling
25 their solubilisation in aqueous media. Numerous applications have been proposed to benefit from this

phenomenon, particularly in the pharmaceutical field, as described by D. Duchêne, in "Pharmaceutical Applications of Cyclodextrins", published in "Cyclodextrins and their industrial uses", Editions de Santé, Paris 1987, pp. 213-257 [1]. Pharmaceutical formulations using cyclodextrins have already been introduced onto the market in Japan, Italy and, more recently, in France, for example by Pierre Fabre Médicament for Brexin® which is an inclusion complex of Piroxicam in β -cyclodextrin.

Of the cyclodextrins that may be used, β -cyclodextrin (comprising 7 glucose units) is the most suitable in terms of the size of its cavity and is the least expensive of the three. Chemical modifications of β -cyclodextrin have been described to render it amphiphilic with a view to incorporating it in organised systems.

In this way, amphiphilic cyclodextrins comprising multiple chains on the primary face have been studied. A. Yabe et al described in "Thin Solid Films", (1988), 160, pp. 33-41 [2], the derivative per(6-dodecylamino-6-deoxy)- β -cyclodextrin in order to form stable Langmuir-Blodgett layers. Similarly, L. Julien et al described in J. Chem. Soc. Perkin Trans 2, 1993, pp. 1011-1022 [3], β -cyclodextrin derivatives comprising aliphatic chains located in primary and secondary positions, with a view to incorporating said cyclodextrin derivatives in phosphatidylcholine vesicles. Said derivatives are amphiphilic and can be incorporated in the vesicles, but the internal cavity of the cyclodextrin is no longer accessible due to the

significant steric size of the aliphatic chains. Consequently, said derivatives are unable to include hydrophobic molecules, particularly active ingredient molecules.

5 Recently, it was demonstrated by A. Gulik et al in Langmuir (1998), 14, pp. 1050-1057 [4], that so-called "skirted" cyclodextrins, comprising fatty acid chains grafted onto secondary hydroxyls could form stable nanospheres. These molecular super-assemblies appear to
10 show very promising encapsulation and releasing properties due to the cumulative effects of the specificity of cyclodextrin size and transport, firstly, and nanoparticle organisation, secondly. However, it is necessary to note that the synthesis and
15 particularly the purification of such cyclodextrins remain very difficult and require long purification steps resulting in low yields. It is clear that the supramolecular organisation properties are drastically linked with the chemical purity of the amphiphilic
20 cyclodextrin derivatives.

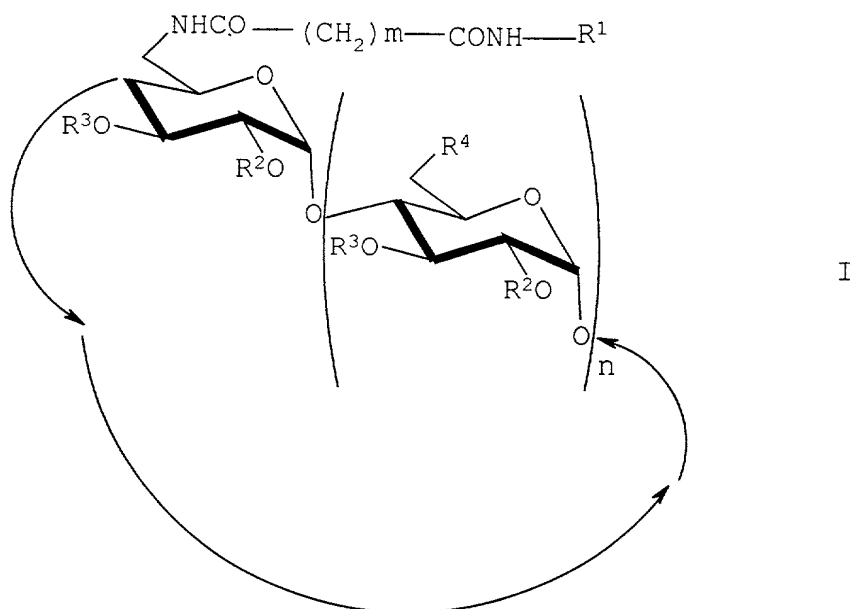
 J. Line et al described in FR-A-2 736 056 [5] and in J. Chem. Soc. Perkin Trans 2, (1998), pp. 2638-2646 [6], the synthesis of cyclodextrin derivatives referred to as "ball joints" comprising one or more aliphatic
25 chains giving them amphiphilic properties, without inducing a self-inclusion phenomenon of the chain(s) in the cyclodextrin. For this reason, it is possible to obtain from such derivatives inclusion complexes containing a hydrophobic molecule and the incorporation
30 of said complexes in phospholipid vesicles. However, these molecules have proved to be relatively unstable

in physiological media, i.e. at pH values greater than or equal to 7, and their incorporation abilities in organised systems remain limited. In addition, said "ball joint" molecules do not organise themselves spontaneously in aqueous media to give particles of well-defined size and shape.

Description of the invention

The present invention specifically relates to amphiphilic cyclodextrin derivatives, stable in physiological media, able to include hydrophobic compounds, having good incorporation abilities in organised systems and also showing self-organisation properties in aqueous media.

According to the invention, the amphiphilic cyclodextrin derivative complying with the formula:



wherein:

- R^1 represents a group derived from a steroid,

- R^2 represents an alkyl or aryl group, substituted if applicable,

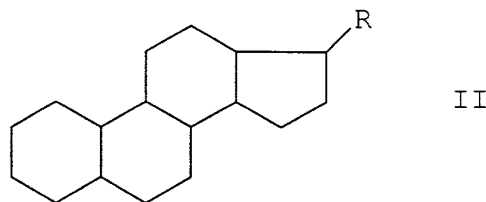
- R^3 represents H or R^2 ,

- all the R^4 represent OR^2 , or

5 - one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2 provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

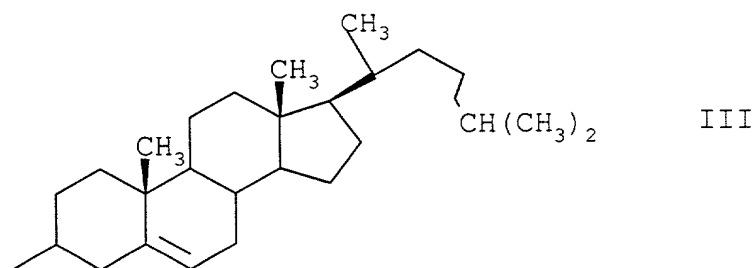
10 - m is an integer ranging from 1 to 8, and
 - n is equal to 5, 6 or 7.

Note that steroids are compounds derived from a polycyclic nucleus according to the formula:



wherein R represents a linear or ramified
 15 hydrocarbon group of 1 to 9 carbon atoms and wherein the polycyclic nucleus may comprise one or more double bonds, and one or more substituents chosen from CH_3 , OH and O, on one or more carbon atoms of the cycles.

In the cyclodextrin derivative according to the
 20 invention, R^1 may represent a group derived from sterols by eliminating the hydroxyl group from the first cycle, with a degree of unsaturation of 0 to 6. It may also consist of groups derived from sterones. For example, R^1 may represent a group derived from
 25 cholesterol such as the group according to the formula:



In the derivative according to the invention, the amphiphilic properties are obtained due to the presence of one or two substituents comprising a group derived from a steroid.

- 5 If the derivative comprises two substituents of this type, it is necessary for them not be located on two adjacent glucose units of the cyclodextrin, due to their size.

10 In addition, the two glucose units comprising said substituents are separated by one or two glucose units comprising an OR^2 substituent.

Preferentially, the cyclodextrin derivative only comprises a single substituent of this type, with all the R^4 representing OR^2 .

- 15 In the cyclodextrin derivative according to the invention, the R^2 group represents a linear or ramified alkyl or aryl group, substituted if applicable. If an alkyl group is used, said group generally has 1 to 4 carbon atoms and is preferentially linear. The aryl
- 20 group may be, for example, the phenyl group or the benzyl group. Any substituents of said alkyl or aryl groups may be, for example, halogen atoms and hydroxyl, carboxyl and amine groups. Advantageously, R^2 represents the methyl group.

R^3 may represent a hydrogen atom or an alkyl group identical or different to R^2 . Preferentially, R^3 represents H.

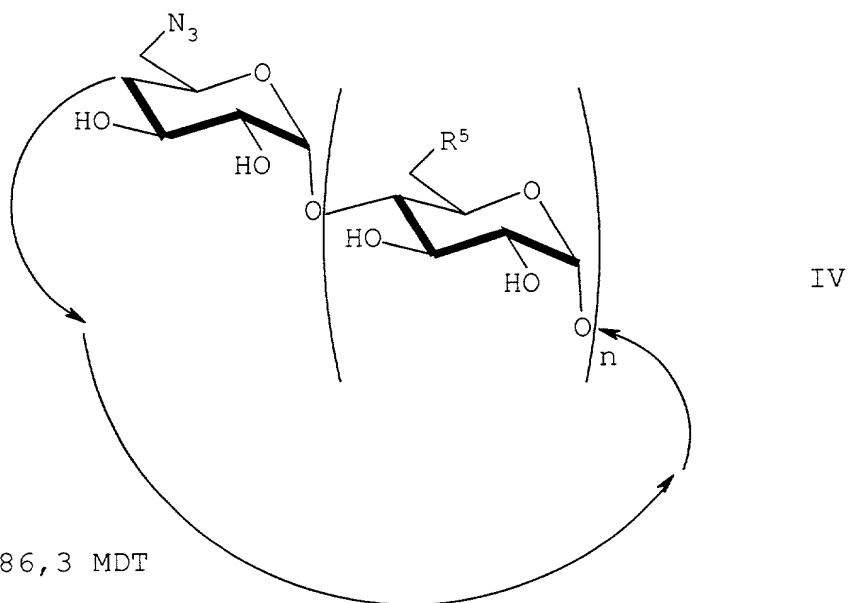
In formula I given above, the aliphatic chain linking the steroid-derived group to the glucose unit may comprise between the two amide groups, of 1 to 8 carbon atoms. Satisfactory results are obtained with two carbon atoms, i.e. where m is equal to 2.

The cyclodextrin derivatives according to the invention may be α -, β - or γ -CD derivatives. Preferentially, β -CD derivatives are used, which corresponds in formula I given above to the case where n is equal to 6.

The cyclodextrin derivatives according to the invention may be prepared using conventional methods from the corresponding mono-azide or diazide cyclodextrin derivatives.

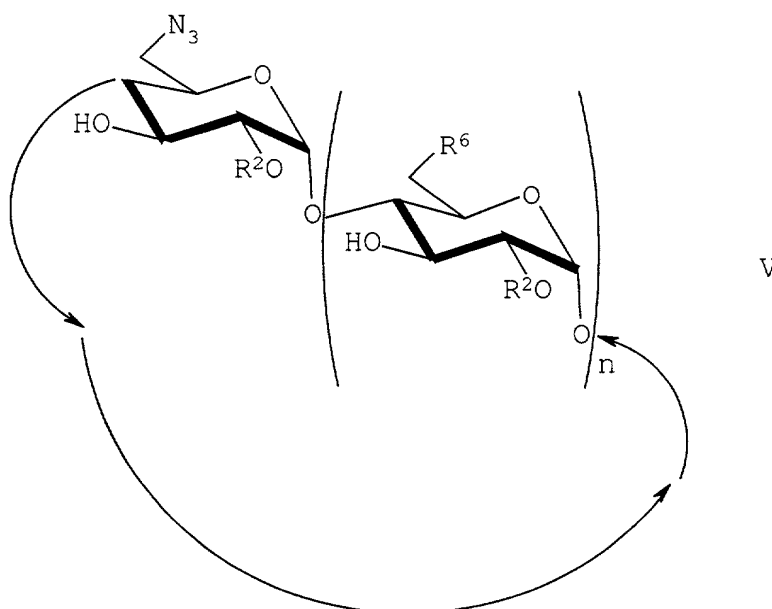
If it is desired to prepare a derivative according to formula I as defined above where R^3 represents a hydrogen atom, the method comprises the following steps:

a) react a derivative according to the formula:



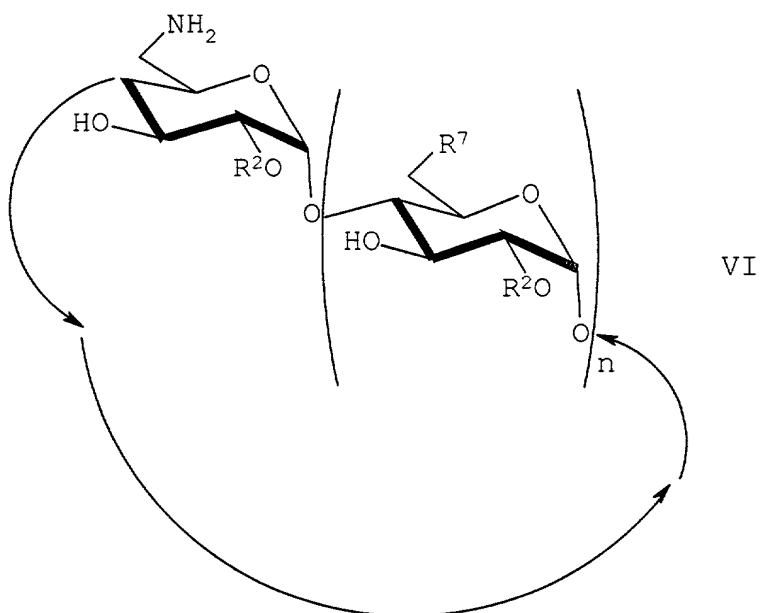
wherein all the R^5 represent OH, or one of the R^5 represents $-N_3$ and the other R^5 represent OH, provided that there is at least one glucose unit where R^5 represents OH between the two glucose units comprising the N_3 substituent, and n is equal to 5, 6 or 7,

with a dialkyl sulphate $SO_4R^2_2$ where R^2 has the significance given above, in a basic medium to obtain the cyclodextrin derivative according to the formula:



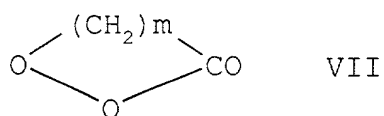
wherein all the R^6 represent OR^2 , or one of the R^6 represents N_3 and the other R^6 represent OR^2 , and R^2 and n are as defined above,

b) perform a Staudinger reaction on the derivative according to formula V using triphenylphosphine and ammonia to convert N_3 into NH_2 and obtain the derivative according to the formula:

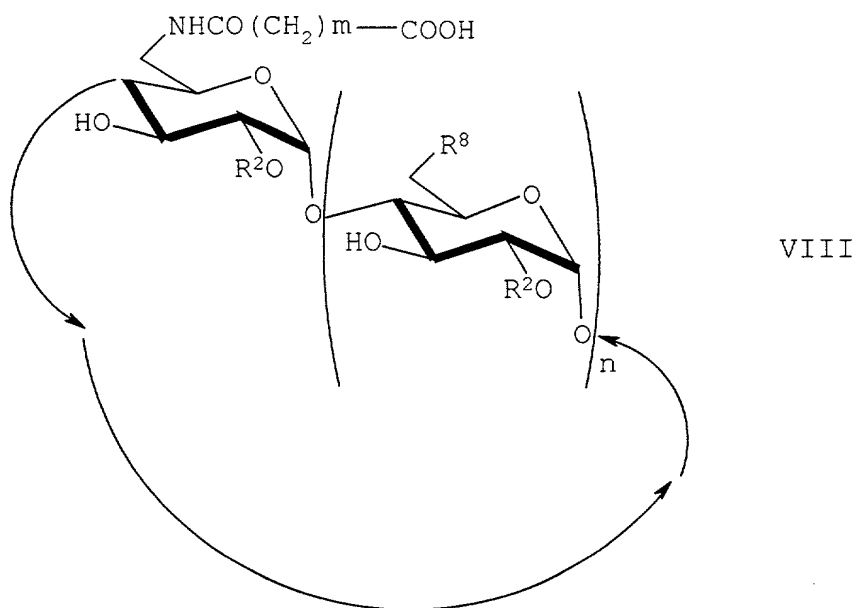


wherein all the R^7 represent OR^2 , or one of the R^7 represents NH_2 and the other R^7 represent OR^2 , and R^2 and n are as defined above,

c) react the derivative according to formula VI
5 with an acid anhydride according to the formula:



where m is as defined above, to obtain the derivative according to the formula:



wherein all the R^8 represent OR^2 , or one of the R^8 represents $-NHCO-(CH_2)_m-COOH$ and the other R^8 represent OR^2 , and R^2 , m and n are as defined above, and

d) react the derivative according to formula VIII
5 with a compound according to the formula NH_2-R^1 to obtain the cyclodextrin derivative according to formula I defined above.

The monoazide or diazide derivatives used as the starting product in the method may be obtained from the
10 corresponding monotosylate or ditosylate cyclodextrin derivative through the action of lithium nitride in water.

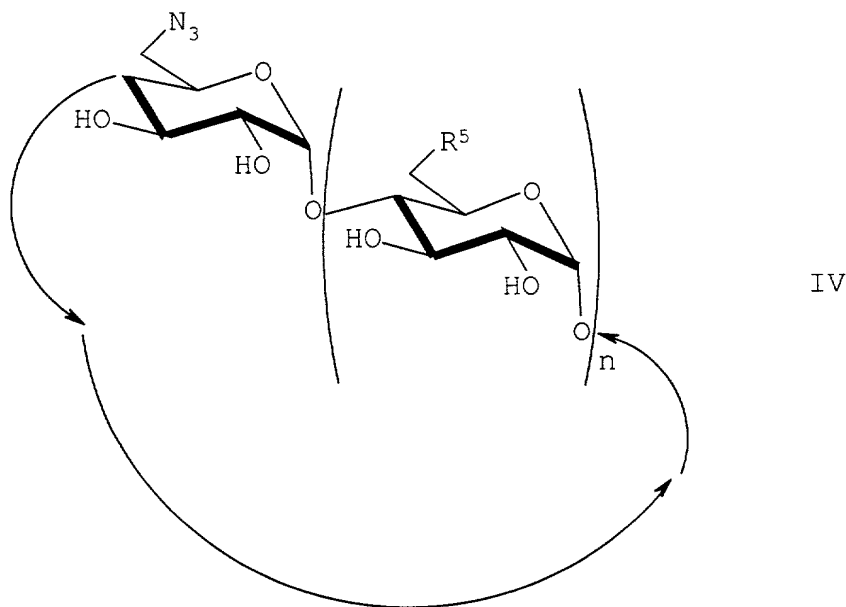
In step a of the method described above, the cyclodextrin derivative according to formula IV is
15 reacted with a dialkyl sulphate $SO_4R^2_2$ in a mixture of organic solvents such as dimethylformamide DMF and dimethylsulphoxide DMSO in 50:50 proportions by volumes, in the presence of a base such as barium oxide and barium hydroxide, at $8^\circ C$. The derivative according
20 to formula V obtained in this way may be separated using the methods described in detail in example 1.

In step b, the derivative according to formula V is reacted with triphenylphosphine in an organic solvent such as DMS and 20% ammonia is then added. The
25 derivative according to formula VI obtained in this way may be purified by evaporating the solvent, eliminating the white precipitate formed by filtration and then separating by ion exchange chromatography. In step c, the derivative according to formula VI is reacted with
30 the acid anhydride according to formula VII desired in an organic solvent such as DMF. The derivative

according to formula VIII obtained is not isolated and the next step d is carried out directly in the same reaction medium. Peptide coupling reagents, such as N,N'-diisopropylcarbodiimide and hydroxybenzotriazole, are then added. The derivative according to formula VIII then reacts with the compound according to the formula H_2N-R^1 such as cholest-5-en-3 α -ylamine. The derivative according to formula I obtained in this way may be separated from the reaction medium by evaporating the solvent and purifying by silica gel column chromatography.

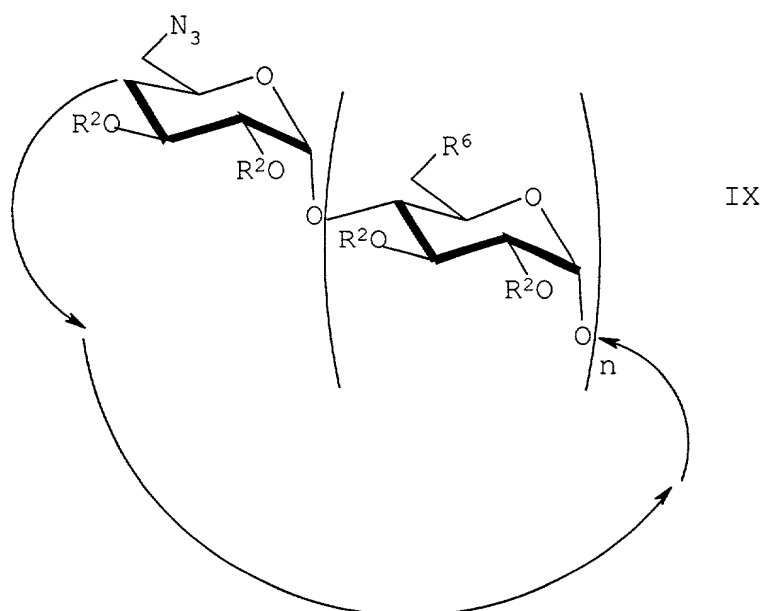
If it is necessary to prepare a derivative according formula I as defined above, where R^3 represents R^2 , the method comprises the same steps as above, but in step a, alkylation of all the OH groups is performed with an iodoalkane. In this case, the following steps are carried out:

a) react a derivative according to the formula:



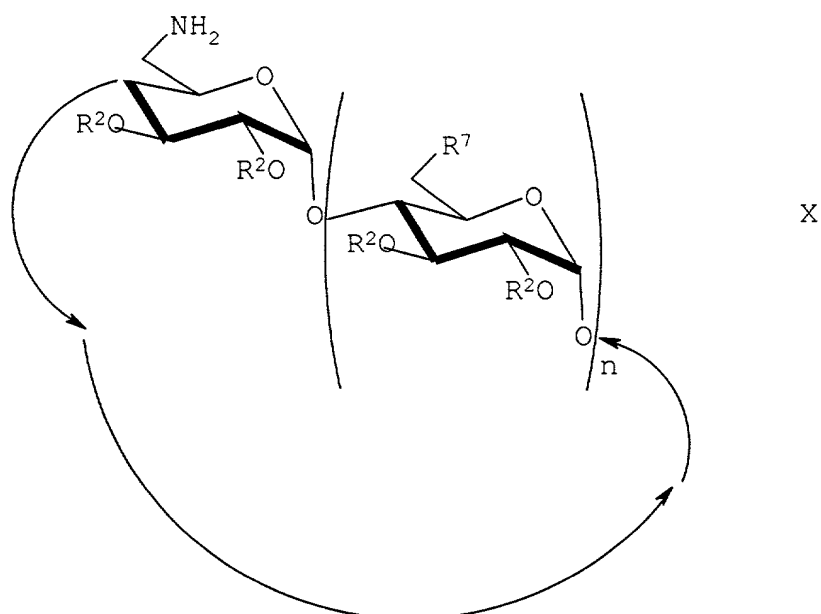
wherein all the R^5 represent OH, or one of the R^5 represents $-N_3$ and the other R^5 represent OH, provided that there is at least one glucose unit where R^5 represents OH between the two glucose units comprising the N_3 substituent, and n is equal to 5, 6 or 7,

with an iodoalkane according to the formula IR^2 wherein R^2 has the significance given above, in the presence of NaH to obtain the cyclodextrin derivative according to the formula:



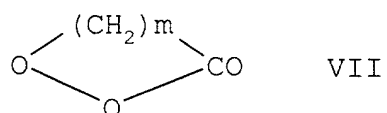
wherein all the R^6 represent OR^2 , or one of the R^6 represents N_3 and the other R^6 represent OR^2 , and R^2 and n are as defined above,

b) perform a Staudinger reaction on the derivative according to formula IX using triphenylphosphine and ammonia to convert N_3 into NH_2 and obtain the derivative according to the formula:

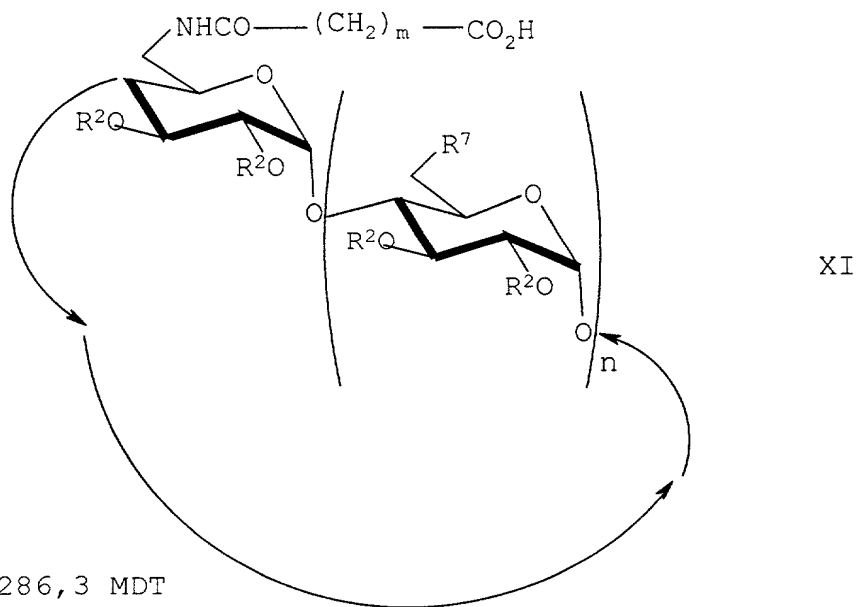


wherein all the R^7 represent OR^2 , or one of the R^7 represents NH_2 and the other R^7 represent OR^2 , and R^2 and n are as defined above,

- 5 c) react the derivative according to formula X with an acid anhydride according to the formula:



where m is as defined above, to obtain the derivative according to the formula:



wherein all the R^7 represent OR^2 , or one of the R^7 represents $-NHCO-(CH_2)_m-COOH$ and the other R^7 represent OR^2 , and R^2 , m and n are as defined above, and

d) react the derivative according to formula XI
5 with a compound according to the formula NH_2-R^1 to obtain the cyclodextrin derivative according to formula I defined above.

The invention also relates to the inclusion
complexes of the cyclodextrin derivative according to
10 formula I with a hydrophobic compound in an aqueous medium. The hydrophobic chemical compounds liable to be solubilised in aqueous media by means of said cyclodextrin derivatives I may be of different types. Examples of such compounds include cosmetic products,
15 vitamins, pharmaceutically active molecules and molecules used as contrast agents for medical imaging, for example, the compounds described by Uekama and Irie in Chemical Review (1998), 98, pp. 2045-2076 [7].

Preferentially in the invention, the hydrophobic
20 chemical compound is a pharmaceutically active molecule. Examples of such molecules include steroids, for example prednisolone, neurotropes such as dothiepin, bacteriostatics such as chloramphenicol, vitamins such as vitamin A, vascular wall tonics such
25 as esculin, and contrast agents for medical imaging such as 16-iodo-3-methylhexadecanoic acid.

Said inclusion complexes may be prepared using
conventional methods, for example by adding to a
solution or suspension of the formula I cyclodextrin
30 used, a solution of hydrophobic compound in a suitable organic solvent, for example acetone.

The formula I cyclodextrin derivatives are characterised in that they organise themselves spontaneously in aqueous media to give nanoparticles from 25 to 30 Å of mean radius and perfectly spherical in shape. The mean number of monomers is 24 cyclodextrin derivative molecules per nanoparticle. In addition, the invention also relates to an aqueous solution of nanoparticles of a cyclodextrin derivative according to formula I alone or in the form of an inclusion complex with a hydrophobic compound.

Said nanoparticle solution may be prepared by forming an aqueous solution of the cyclodextrin derivative or an inclusion complex of said derivative having a derivative or complex concentration greater than the critical micellar concentration of the derivative.

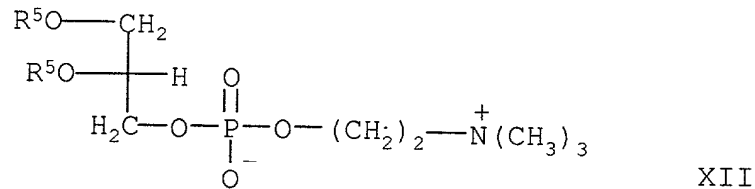
The self-organisation of amphiphilic cyclodextrins into nanoparticles in an aqueous medium makes it possible to transport a hydrophobic molecule, for example an active ingredient, particularly by the transmembrane or parenteral route.

Moreover, the cyclodextrin derivatives according to the invention are of particular interest since they can be incorporated into organised surfactant systems such as small phospholipid vesicles or micellae. Said incorporation is intended to enable the solubilisation of organised systems, with a view to transporting active ingredients included in the cyclodextrin derivative.

In addition, the invention also relates to an organised surfactant system comprising a cyclodextrin

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derivative or an inclusion complex of said derivative according to the invention. The surfactants liable to form such organised systems may be of different types. Examples include the phospholipids complying with the
5 following general formula:



wherein R^5 represents $\text{CH}_3-(\text{CH}_2)_p-\text{CO}$ where p is an integer ranging from 6 to 18. Said phospholipids are capable of forming small unilamellar vesicles. This particularly applies to dimyristoylphosphatidylcholine
10 DMPC which complies with the above formula where $p = 12$.

To incorporate the cyclodextrin derivative or an inclusion complex of said derivative according to the invention in the organised surfactant system, it is
15 possible to form small DMPC vesicles beforehand by sonication and then add in the aqueous solution the cyclodextrin derivative or the inclusion complex. The combined system obtained in this way then becomes perfectly soluble in water, resulting in a clear
20 solution. The combined system obtained in this specific case is a combined micella of a mean radius of 60 Å.

In addition, the invention also relates to an aqueous solution comprising in solution a combined system formed from phospholipid or membrane protein
25 vesicles, and at least one cyclodextrin derivative or at least one cyclodextrin derivative inclusion complex according to the invention.

Such solutions are beneficial since they enable the transport of hydrophobic molecules, for example an active ingredient, by the transmembrane or parenteral route, for pharmaceutical or cosmetic applications.

5 The invention's other characteristics and advantages will be seen more clearly upon reading the following examples, which are naturally illustrative and not exhaustive, with reference to the appended figures 1 to 5.

10

Brief description of figures

Figure 1 illustrates the experimental neutron diffusion spectrum, on a logarithmic scale, of an aqueous solution of nanoparticles of the cyclodextrin derivative obtained in example 1 with three theoretical curves of spherical micellae, cylindrical micellae and double layers.

Figure 2 illustrates the appearance of different cyclodextrin and phospholipid mixtures.

20 Figures 3, 4 and 5 illustrate the ^{31}P nuclear magnetic resonance spectra obtained from sample a (figure 3), b (figure 5) and d (figure 4), respectively, in example 3.

Figure 6 illustrates the experimental neutron diffusion spectrum, on a logarithmic scale, of a DMPC/cyclodextrin derivative mixture (sample d) obtained in example 3 with three theoretical curves of spherical micellae, cylindrical micellae and double layers.

30 Figure 7 illustrates the experimental neutron diffusion spectrum, on a logarithmic scale, of mixtures

of 16-iodo-3-methylhexadecanoic acid and the cyclodextrin derivative in example 1 (1/1 and 0.1/1 mol) and the neutron diffusion spectrum of the cyclodextrin derivative in example 1 alone with the theoretical diffusion curve of nanoparticles alone.

Detailed description of embodiments

Example 1: Synthesis of mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose.

This compound is the derivative according to formula I where R¹ represents the group according to formula III, R² is the methyl group, R³ represents H, all the R⁴ represent OCH₃, m is equal to 2 and n is equal to 6.

a) Preparation of mono-6-azide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose.

In a flask, 2 g (1.7 mmol) of mono-6-azide-6-deoxy-cyclomaltoheptaose (obtained for example according to the protocol described in Tetrahedron Lett. (1993), 34, pp. 2457-2460 [8] and J. Chem. Soc. Perkin Trans 2 (1995), pp. 723-730 [9]) is dissolved in 15 ml of anhydrous dimethylsulphoxide. This solution is supplemented with 15 ml of anhydrous dimethylformamide DMF. In a nitrogen atmosphere and under vigorous stirring, 3.8 g (~12 mmol) of barium hydroxide octohydrate and 3.6 g (~24 mmol) of barium oxide are then added. After homogenising the medium, 8

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ml of dimethyl sulphate (~84 mmol) is added, and the mixture is left under vigorous stirring, in a nitrogen atmosphere, for 30 hours at 8°C. The milky suspension is then supplemented with 5 ml of 20% ammonia and stirred for 3 hours at ambient temperature. The suspension is allowed to settle overnight in a refrigerator. After concentrating the supernatant at reduced pressure, the residual solid is taken up with 100 ml of dichloromethane, and two more times with 50 ml of dichloromethane. The organic phases are pooled, washed 3 times with 20 ml of an aqueous solution saturated with sodium chloride, twice with 20 ml of water, dried on magnesium sulphate and concentrated at reduced pressure. The product is precipitated by adding 100 ml of n-hexane, filtered, washed with 100 ml of n-hexane and dried in a vacuum.

0.80 g (0.60 mmol) of mono-6-azide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose, which is presented in the form of a white powder, is collected.

b) Preparation of mono-6-amino-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose

0.75 g (0.56 mmol) of the compound obtained in a is dissolved in 30 ml of DMF. To this solution, 0.75 g (2.86 mmol) of triphenylphosphine in 5 ml of DMF is added drop by drop, under stirring at ambient temperature. The reaction medium is maintained at ambient temperature for 2 hours, cooled to 0°C and treated with 14 ml of 20% ammonia. It is left for 18

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hours at ambient temperature under stirring, and the solvent is then eliminated at reduced pressure and the residual solid is taken up with 30 ml of water. The insoluble excess of triphenylphosphine and the
5 corresponding oxide is eliminated by filtration. The solution is concentrated in a vacuum and the product is purified by ion exchange resin column chromatography (Lewatit® SP 1080 resin in H⁺ form). 0.35 g (0.27 mmol) of
10 mono-6-amino-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6'''''-trideca-O-methyl-cyclomaltoheptaose, which is presented in the form of a white powder, is collected.

c) Preparation of mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6'''''-trideca-O-methyl-cyclomaltoheptaose.
15

To a solution of 0.25 g (0.19 mmol) of the compound obtained in b in 6 ml of anhydrous DMF, 0.019 g (0.19 mmol)
20 of succinic anhydride in 2 ml of anhydrous DMF is added in a nitrogen atmosphere under stirring. The reaction medium is maintained at ambient temperature for 5 hours, and then supplemented with 0.11 ml (0.76 mmol) of N,N'-diisopropylcarbodiimide and 0.028 g (0.19 mmol) of
25 hydroxybenzotriazole in 2 ml of anhydrous DMF. After 30 minutes of stirring at ambient temperature, 0.089 g (0.23 mmol) of cholest-5-en-3 α -ylamine (obtained in two steps from cholest-5-en-3 β -ol according to the protocols described in Tetrahedron Lett. (1977), pp. 1977-1980 [10]) is added. The
30 reaction medium is left under stirring at ambient temperature for 48 hours, hydrolysed by adding 0.30 ml

of water and concentrated under reduced pressure. The residual solid is purified by silica gel column chromatography (60 Fluka silica gel; eluent: CH₂Cl₂-MeOH 95:5 followed by 9:1 (v/v)). 0.24 g of mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''''',2''''''',6',6'',6''',6''''',6''''''',6'''''''-trideca-O-methyl-cyclomaltoheptaose is collected.

(71% final compound yield from mono-6-amino-6-deoxy-2,2',2'',2''',2''''',2''''''',2''''''',6',6'',6''',6''''',6''''''',6'''''''-trideca-O-methyl-cyclomaltoheptaose).

The characteristics of this compound are as follows:

- Thin layer chromatography (Merck Silica Plates)
15 Rf = 0.50 in the 9:1 (v/v) CH₂Cl₂-MeOH mixture, detection with 10% H₂SO₄.

- Mass spectrometry: ESI-MS: m/z = 1805.95 [M+Na]⁺ for C₈₆ H₁₄₆ N₂O₃₆Na.

- NMR ¹H (500 MHz, 25°C, 7 mM solution in CDCl₃):
20 attribution by COSY and relay COSY experiments: δ = 6.49 (NH CD), 5.70 (NH Chol), 5.38 (H-6 Chol), 5.28-4.88 (H-1, OH-3 CD), 4.11 (H-3 Chol), 3.99-3.16 (H-2, H-3, H-4, H-5, H-6, H-6', OCH₃ CD), 2.60-2.48 (CH₂ succ, H-4 Chol), 2.04-0.68 (H Chol).

25

Example 2: Preparation of mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''''',2''''''',6',6'',6''',6''''',6''''''',6'''''''-trideca-O-methyl-cyclomaltoheptaose nanoparticles.

30 The mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''''',2''''''',6',6'',

6''',6''',6''',6''''-trideca-O-methyl-
cyclomaltoheptaose nanoparticles are prepared simply by
forming an aqueous solution of this cholesteryl-
cyclodextrin at a concentration greater than its
5 critical micellar concentration cmc.

The cmc of the cyclodextrin in example 1 was
determined with surface tension measurements. The value
of the cmc is $9 \cdot 10^{-6}$ mol/l.

The mean hydrodynamic diameter of the
10 nanoparticles was measured by quasi-elastic light
diffusion. The mean diameter MD value calculated
according to the Stokes-Einstein approximation based on
the interaction-free perfect sphere model is 0.6 nm (60
Å). The static light diffusion analysis of aqueous
15 solutions of nanoparticles at different concentrations
($2.5 \cdot 10^{-3}$, $5 \cdot 10^{-3}$ and 10^{-2} mol/l) gives a mean aggregate
mass of 43,000 g/mol, which is equivalent to a mean of
24 monomers per nanoparticle.

The perfectly spherical shape and the size of the
20 nanoparticles were confirmed by neutron diffusion. The
diffusion spectrum obtained from a 10^{-2} mol/l solution
in D₂O of the cyclodextrin derivative in example 1 is
represented in figure 1 (spectrum 1).

In figure 1, the theoretical spectra simulating
25 spheres (spectrum 2), cylinders (spectrum 3) or
lamellae (spectrum 4) formed from the cyclodextrin
derivative in example 1, at a concentration of 10^{-2}
mol/l in D₂O, are also shown. Superimposing spectrum 2
simulating spheres with experimental spectrum 1 proves
30 the spherical shape of the cholesteryl-cyclodextrin
aggregates. These aggregates are lined on the surface

with cyclodextrin cavities available for the inclusion of hydrophobic active molecules, the core being composed of cholesterol groups. The theoretical spectrum simulating spheres gives a mean diameter of 0.5 nm (50 Å) and a mean number of monomers per nanoparticle of 24.

Example 3: Preparation of combined mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose and Di-Myristoyl-Phosphatidyl-Choline DMPC systems.

An aqueous $15 \cdot 10^{-3}$ mol/l DMPC solution is prepared either in the form of multilamellar large vesicles MLVs, or in the form of small unilamellar vesicles SUVs, by following the preparation protocols described for example in "Liposomes: a practical approach", R.R.C. New Ed., IRL Press, Oxford University Press, 1990 [11]. To the DMPC MLV or SUV suspension, the cyclodextrin in example 1 is added such that the final cyclodextrin concentration in the aqueous cyclodextrin/DMPC mixture is $0.5 \cdot 10^{-3}$ or $2.5 \cdot 10^{-3}$ mol/l.

Figure 2 illustrates the appearance of the different mixtures after 12 hours at 25°C. Tube a contains an aqueous suspension of 15 mM DMPC unilamellar vesicles. Tubes c and d correspond to the following DMPC/cyclodextrin mixtures: $15 \cdot 10^{-3}/0.5 \cdot 10^{-3}$ and $15 \cdot 10^{-3}/2.5 \cdot 10^{-3}$ mol/l respectively. Tube b is a "control" tube corresponding to the $15 \cdot 10^{-3}/2.5 \cdot 10^{-3}$ mol/l DMPC/heptakis(2,6-di-O-methyl)cyclomaltoheptaose

mixture, i.e. a mixture of DMPC with a cyclodextrin comprising no steroid substituent.

The different mixtures are examined by ^{31}P NMR spectroscopy at 81 MHz.

5 Figure 3 represents the spectrum corresponding to tube a (DPMC only).

Figure 4 represents the spectrum corresponding to tube d (DMPC/cyclodextrin mixture; $15.10^{-3}/2.5.10^{-3}$ mol/l).

10 Figure 5 represents the spectrum corresponding to tube b (DMPC/cyclodextrin mixture according to the prior art).

The existence of small unilamellar DMPC vesicles in tube a is confirmed on the spectrum in figure 3 by
15 the presence of two very thin peaks at around 0 ppm corresponding to the phosphors located inside and outside the vesicles.

The spectrum in figure 4, which corresponds to sample d, which is perfectly transparent, is reduced to
20 a single thin peak centred at 0 ppm, indicating the presence of smaller aggregates than the unilamellar vesicles in tube a. The spectrum corresponding to the "control" tube b indicates the formation of larger vesicles than the initial vesicles. There is no
25 reorganisation of the medium with this cyclodextrin.

In tube c, the quantity of cyclodextrin according to the invention is too low with reference to the quantity of DMPC to produce a transparent solution as in tube d. A two-phase mixture is obtained.

30 The mean hydrodynamic diameter of the combined aggregates in sample d was measured by quasi-elastic

light diffusion. The mean diameter MD value calculated according to the Stokes-Einstein approximation based on the interaction-free perfect sphere model is 13 nm (130 Å).

5 Sample d was then examined by neutron diffusion.

Figure 6 illustrates the diffusion spectrum obtained (spectrum 5). In figure 6, the theoretical spectra simulating spheres (spectrum 6), cylinders (spectrum 7) or lamellae (spectrum 8) formed from the cyclodextrin derivative in example 1, at a ratio of 10 15.10⁻³/2.5.10⁻³ mol/l in D₂O, are also shown. Superimposing spectrum 6 simulating spheres with experimental spectrum 5 proves the spherical shape of the combined aggregates of DMPC/cyclodextrin according to example 1. These combined spherical systems comprise on their surface cyclodextrin cavities available for the inclusion of hydrophobic active molecules. The theoretical spectrum simulating spheres gives a mean diameter of 10.8 nm (108 Å)

20

Example 4: Preparation of inclusion complexes of the mono-6-(cholest-5-en-3α-ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose
25 compound with various hydrophobic active molecules.

To an aqueous solution of mono-6-(cholest-5-en-3α-ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose nanoparticles
30 obtained as in example 2, the hydrophobic compound is simply added either directly, or in solution in a

suitable organic solvent, for example acetone, which is allowed to evaporate slowly in air.

Various hydrophobic active molecules were tested and proved to be capable of forming inclusion complexes with the cyclodextrin in example 1. In this way, 16-iodo-3-methylhexadecanoic acid, a fatty acid used as a contrast agent for medical imaging, which had already been solubilised in cyclodextrins, as described in FR-A-2 726 765 [12], was solubilised in an aqueous medium by forming an inclusion complex.

Figure 7 represents the neutron diffusion spectra obtained with:

- the solution of nanoparticles of cyclodextrin according to example 1 and the fatty acid (1:0.5 eq. mol) (spectrum 9);
- the solution of nanoparticles of cyclodextrin according to example 1 and the fatty acid (1:1 eq. mol) (spectrum 10); and
- the solution of nanoparticles of cyclodextrin alone (spectrum 11).

In this figure, the theoretical spectrum (spectrum 12) simulating spheres has also been represented.

In this figure, it can be seen that the incorporation of fatty acid molecules in the nanoparticles of the cyclodextrin according to example 1 results in marked modifications on the diffusion spectra. The intensity $I(q)$ is increased. The Intensity $I(q)$ is proportional to the volume of nanoparticles. The presence of additional molecules (fatty acid molecules) in the nanoparticles increases the value of the contrast and therefore the intensity.

The incorporation in mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''''',2''''',2''''',6',6'',6''',6''''',6''''',6''''''-trideca-O-methyl-cyclomaltoheptaose nanoparticles of
 5 the following hydrophobic compounds:
 - dothiepin (neurotrope),
 - chloramphenicol (bacteriostatic),
 - vitamin A, and
 - esculin (vascular wall tonic),
 10 were also detected by neutron diffusion.

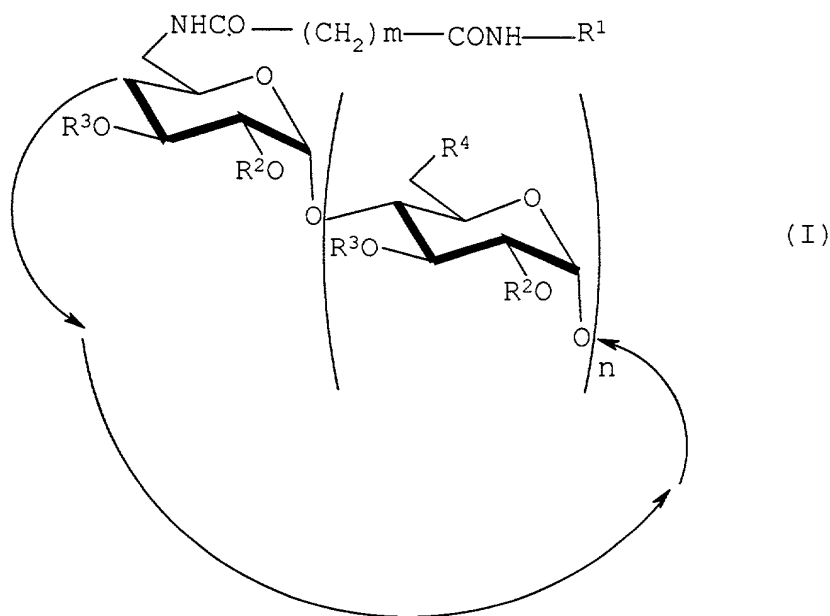
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CLAIMS

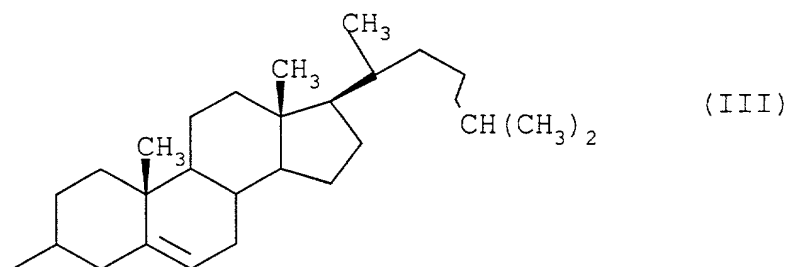
1. Amphiphilic cyclodextrin derivative complying with the formula:



wherein:

- R^1 represents a group derived from a steroid,
- 5 - R^2 represents an alkyl or aryl group, substituted if applicable,
- R^3 represents H or R^2 ,
- all the R^4 represent OR^2 , or
- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and
- 10 the other R^4 represent OR^2 provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,
- m is an integer ranging from 1 to 8, and
- 15 - n is equal to 5, 6 or 7.

2. Cyclodextrin derivative according to claim 1 wherein R^1 represents the group according to the formula:



3. Cyclodextrin derivative according to claim 1 or 2, wherein all the R^4 represent OR^2 .

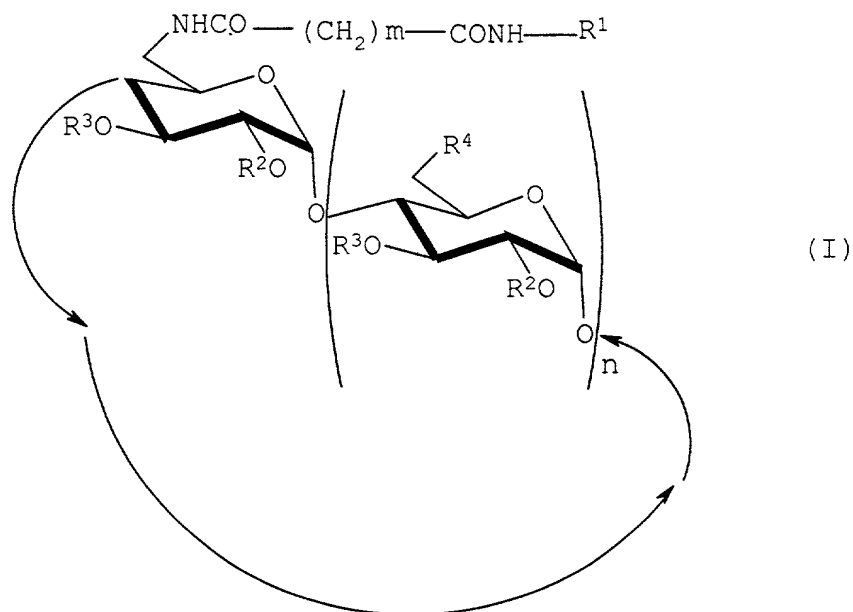
4. Cyclodextrin derivative according to any of claims 1 to 3, wherein R^2 represents the methyl group and R^3 represents a hydrogen atom.

5. Cyclodextrin derivative according to any of claims 1 to 4, wherein n is equal to 6.

6. Cyclodextrin derivative according to any of claims 1 to 5, wherein m is equal to 2.

7. Mono-6-(cholest-5-en-3 α -ylamide)succinylamide-6-deoxy-2,2',2'',2''',2''',2''',2''',2''',6',6'',6''',6''',6''',6''',6''''-trideca-O-methyl-cyclomaltoheptaose.

8. Method to prepare a cyclodextrin derivative according to the formula:



wherein:

- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,

5 - R^3 represents H,

- all the R^4 represent OR^2 , or

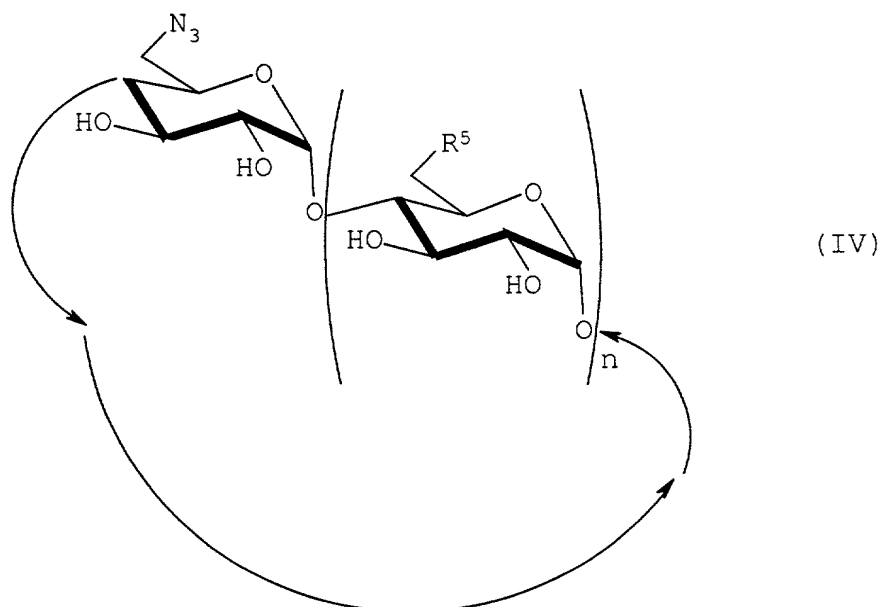
10 - one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2 provided that there is at least one glucose unit where R^4 represents OR^2 between the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

- m is an integer ranging from 1 to 8, and

- n is equal to 5, 6 or 7,

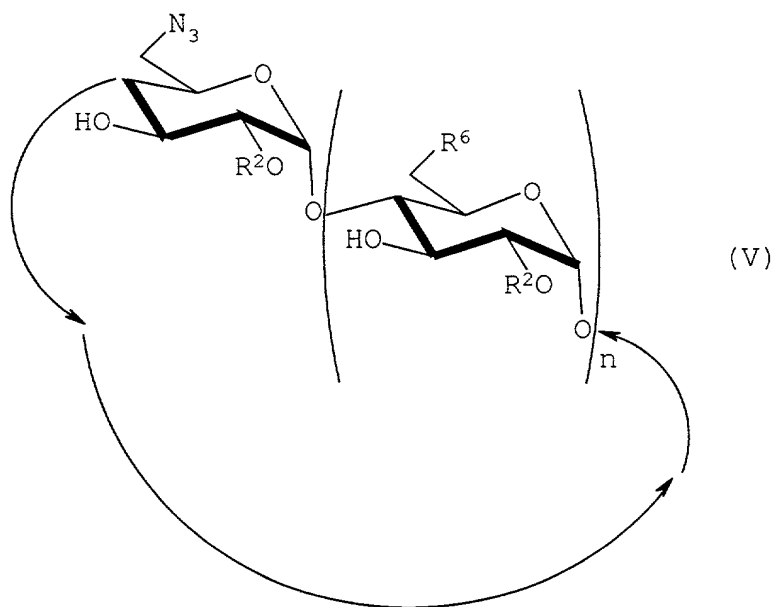
which comprises the following steps:

15 a) react a derivative according to the formula:



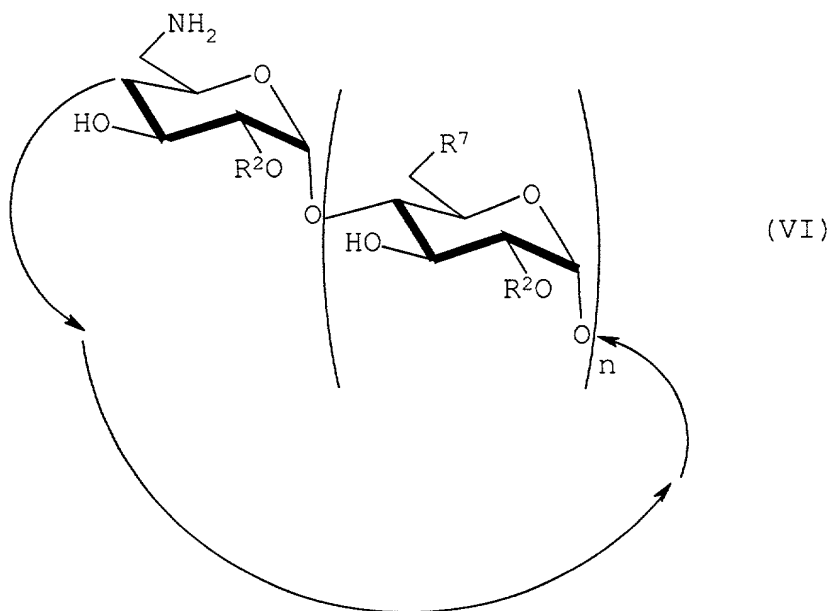
wherein all the R^5 represent OH, or one of the R^5 represents $-N_3$ and the other R^5 represent OH, provided that there is at least one glucose unit where R^5 represents OH between the two glucose units comprising the N_3 substituent, and n is equal to 5, 6 or 7,

with a dialkyl sulphate $SO_4R^2_2$ where R^2 has the significance given above, in a basic medium to obtain the cyclodextrin derivative according to the formula:



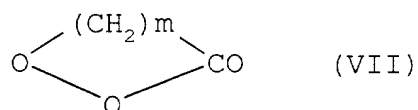
wherein all the R^6 represent OR^2 , or one of the R^6 represents N_3 and the other R^6 represent OR^2 , and R^2 and n are as defined above,

- 5 b) perform a Staudinger reaction on the derivative according to formula (V) using triphenylphosphine and ammonia to convert N_3 into NH_2 and obtain the derivative according to the formula:

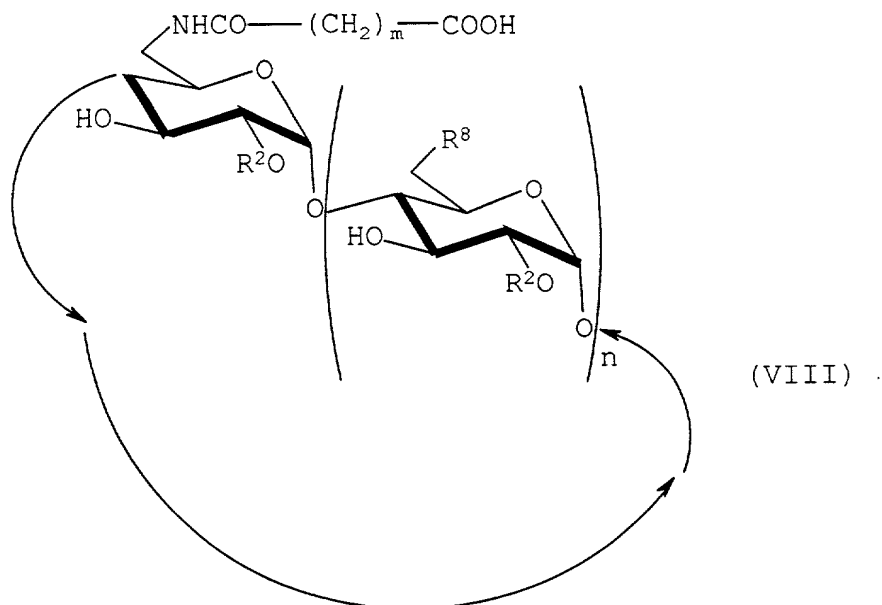


- 10 wherein all the R^7 represent OR^2 , or one of the R^7 represents NH_2 and the other R^7 represent OR^2 , and R^2 and n are as defined above,

c) react the derivative according to formula (VI) with an acid anhydride according to the formula:



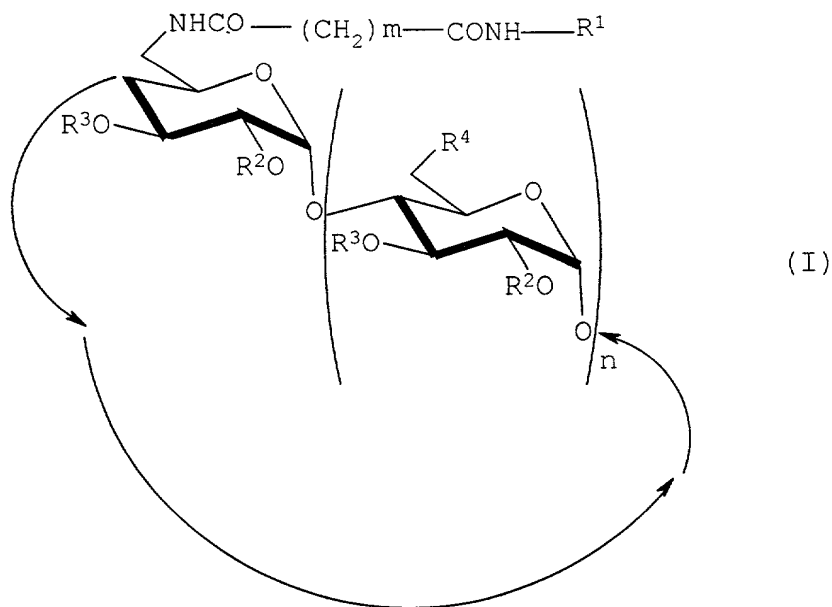
- 15 where m is as defined above, to obtain the derivative according to the formula:



wherein all the R^8 represent OR^2 , or one of the R^8 represents $-NHCO-(CH_2)_m-COOH$ and the other R^8 represent OR^2 , and R^2 , m and n are as defined above, and

- d) react the derivative according to formula 5 (VIII) with a compound according to the formula NH_2-R^1 to obtain the cyclodextrin derivative according to formula (I) defined above.

9. Method to prepare a cyclodextrin derivative
10 according to the formula:



wherein:

- R^1 represents a group derived from a steroid,
- R^2 represents an alkyl or aryl group, substituted if applicable,

5

- R^3 represents R^2 ,

- all the R^4 represent OR^2 , or

- one of the R^4 represents $-NHCO(CH_2)_mCONHR^1$, and the other R^4 represent OR^2 provided that there is at least one glucose unit where R^4 represents OR^2 between

10

the two glucose units comprising the substituent $-NHCO-(CH_2)_m-CONH-R^1$,

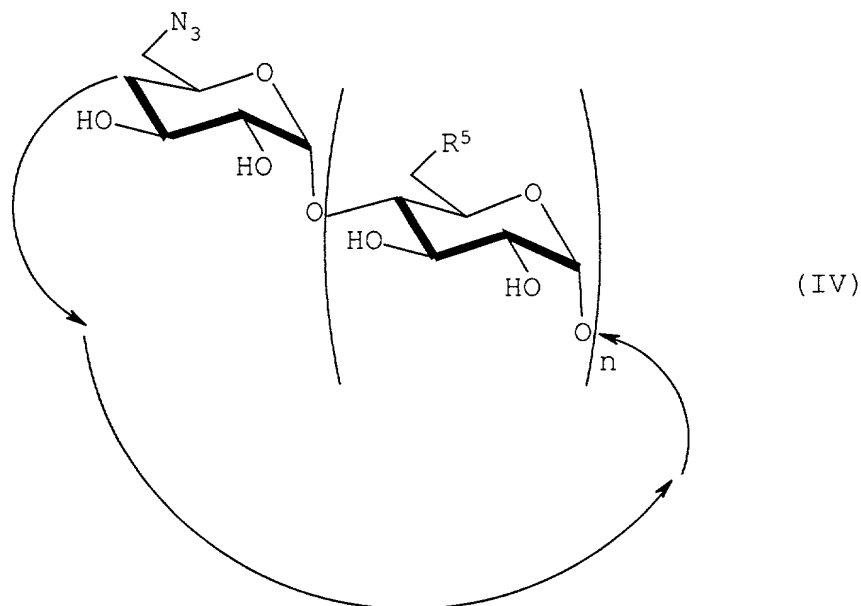
- m is an integer ranging from 1 to 8, and

- n is equal to 5, 6 or 7,

which comprises the following steps:

15

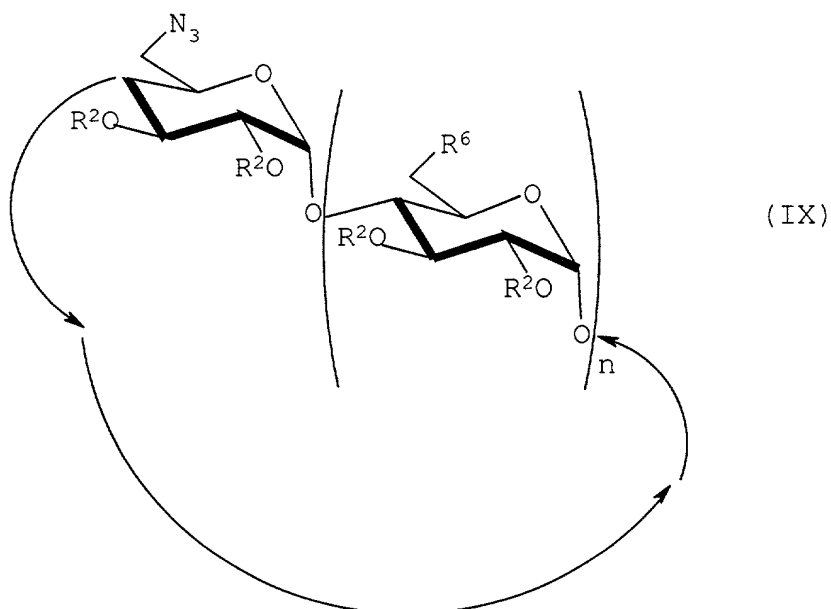
a) react a derivative according to the formula:



wherein all the R^5 represent OH , or one of the R^5 represents $-N_3$ and the other R^5 represent OH , provided that there is at least one glucose unit where R^5

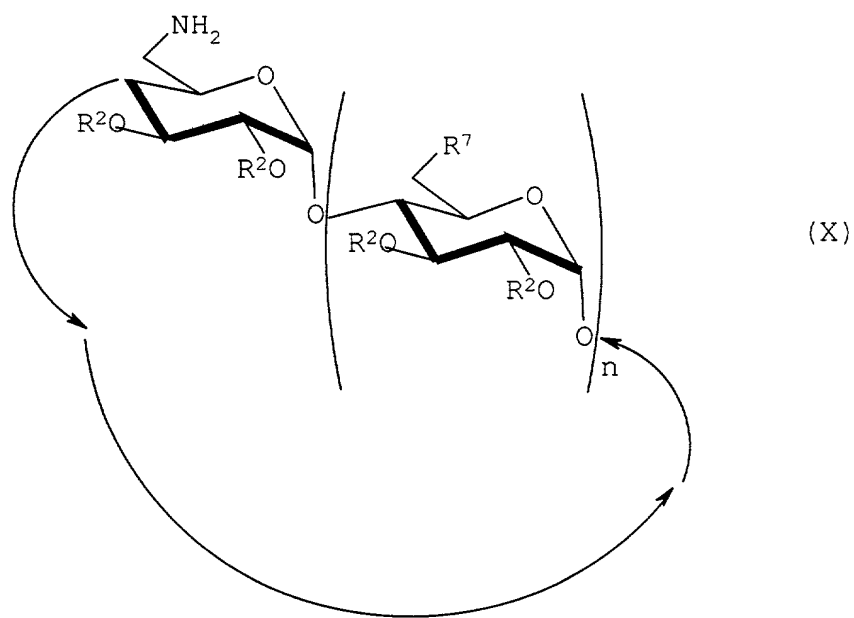
represents OH between the two glucose units comprising the N_3 substituent, and n is equal to 5, 6 or 7,

with an iodoalkane according to the formula IR^2 wherein R^2 has the significance given above, in the presence of NaH to obtain the cyclodextrin derivative according to the formula:



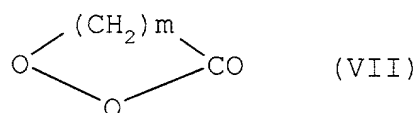
wherein all the R^6 represent OR^2 , or one of the R^6 represents N_3 and the other R^6 represent OR^2 , and R^2 and n are as defined above,

b) perform a Staudinger reaction on the derivative according to formula (IX) using triphenylphosphine and ammonia to convert N_3 into NH_2 and obtain the derivative according to the formula:

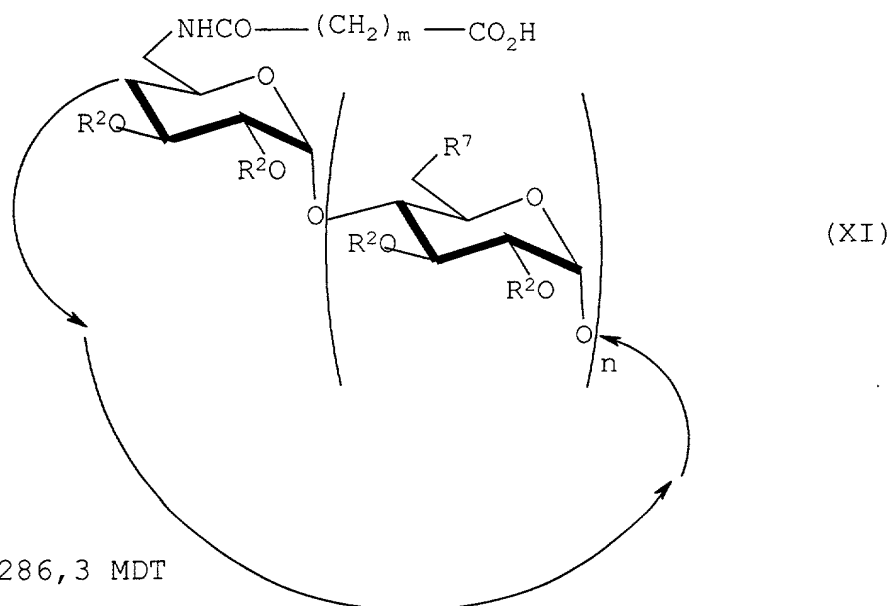


wherein all the R^7 represent OR^2 , or one of the R^7 represents NH_2 and the other R^7 represent OR^2 , and R^2 and n are as defined above,

- c) react the derivative according to formula (X)
 5 with an acid anhydride according to the formula:



where m is as defined above, to obtain the derivative according to the formula:



wherein all the R^7 represent OR^2 , or one of the R^7 represents $-NHCO-(CH_2)_m-COOH$ and the other R^7 represent OR^2 , and R^2 , m and n are as defined above, and

d) react the derivative according to formula (XI)
 5 with a compound according to the formula NH_2-R^1 to obtain the cyclodextrin derivative according to formula (I) defined above.

10 10. Inclusion complex of a cyclodextrin derivative according to any of claims 1 to 7, and a hydrophobic compound.

15 11. Complex according to claim 10, wherein the hydrophobic compound is chosen from steroids, neurotropes, bacteriostatics, vitamins, vascular wall tonics and contrast agents.

20 12. Complex according to claim 10, wherein the hydrophobic compound is chosen from 16-iodo-3-methylhexadecanoic acid, dothiepin, chloramphenicol, vitamin A and esculin.

25 13. Aqueous solution of nanoparticles of a cyclodextrin derivative according to any of claims 1 to 7 or an inclusion complex according to any of claims 10 to 12.

30 14. Organised surfactant system comprising a cyclodextrin derivative according to any of claims 1 to 7 or an inclusion complex according to any of claims 10 to 12.

15. System according to claim 14 wherein the surfactant is a phospholipid.

5 16. Aqueous solution comprising in solution a combined system formed from phospholipid or membrane protein vesicles, and at least one cyclodextrin derivative according to any of claims 1 to 7 or at least one cyclodextrin derivative inclusion complex
10 according to any of claims 10 to 12.

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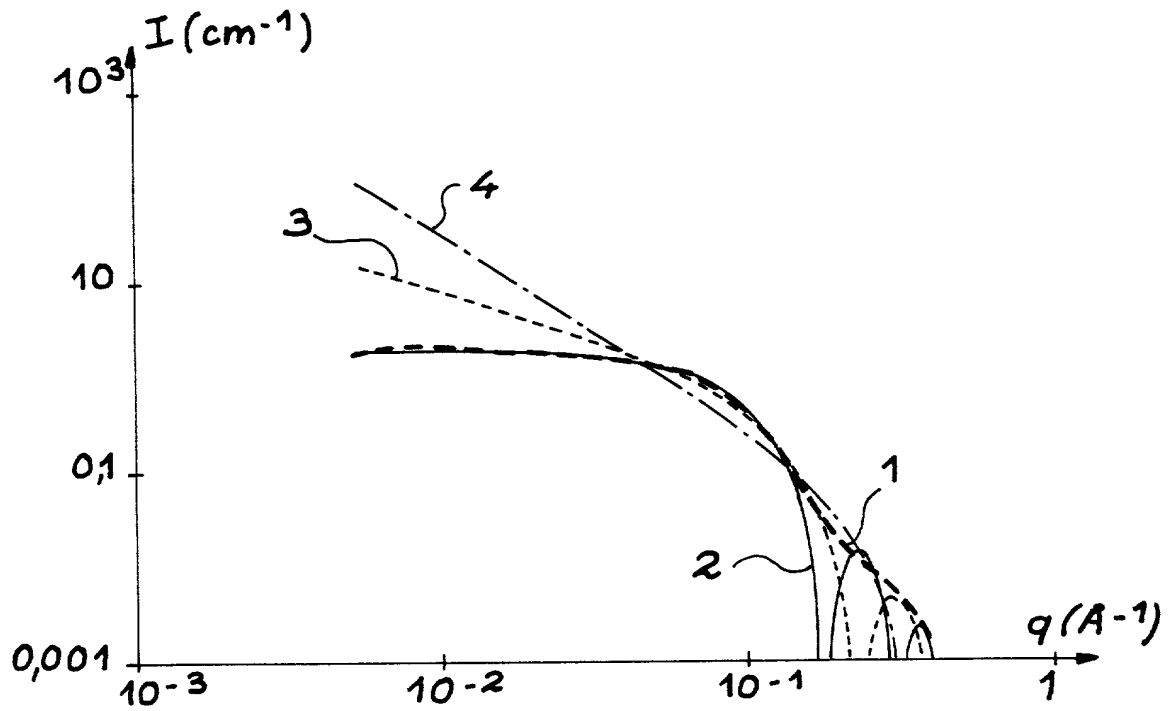


FIG. 1

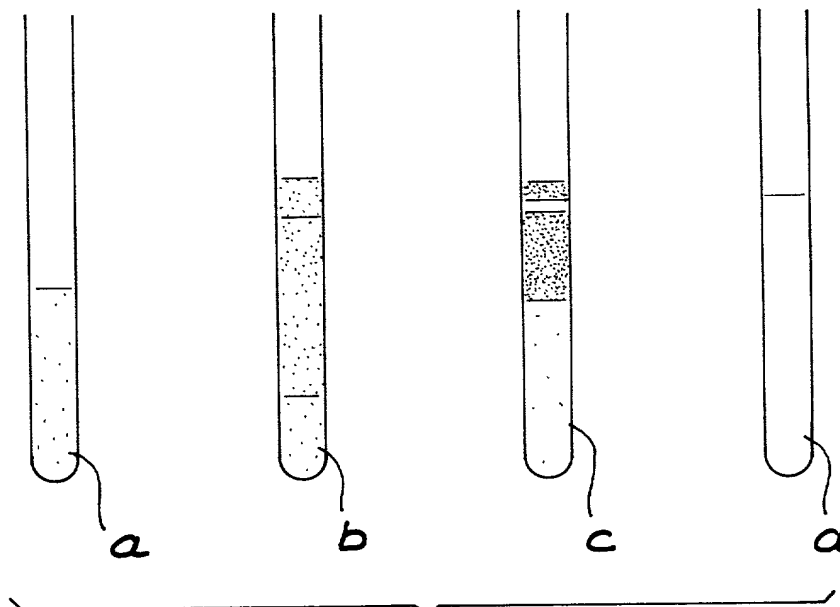


FIG. 2

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FIG. 3

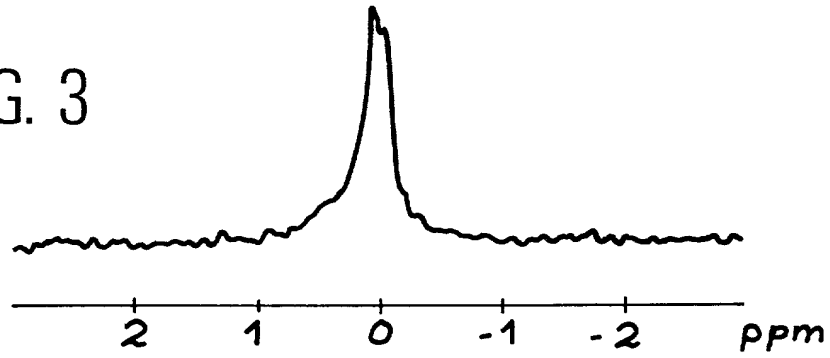


FIG. 4

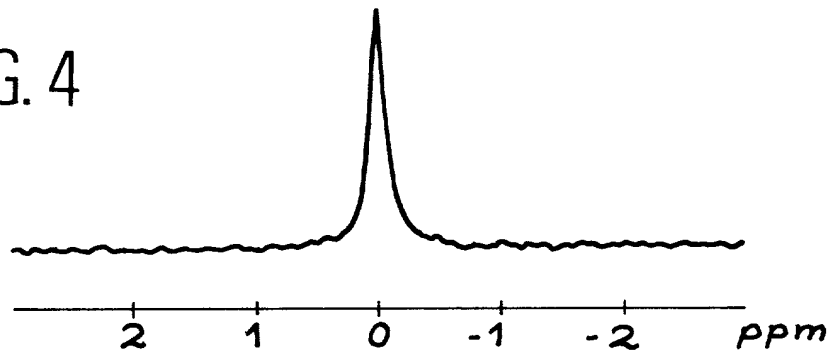
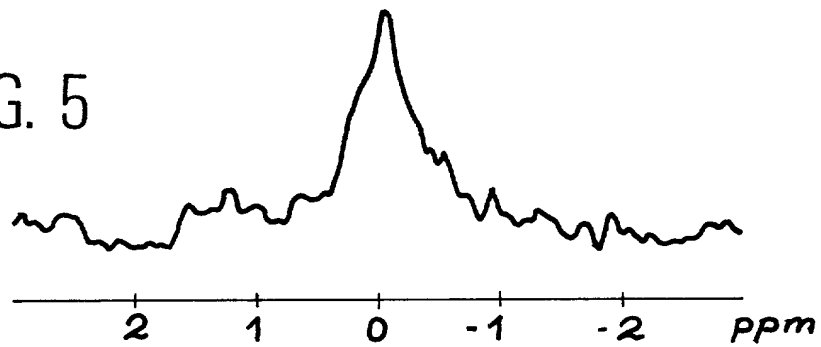


FIG. 5



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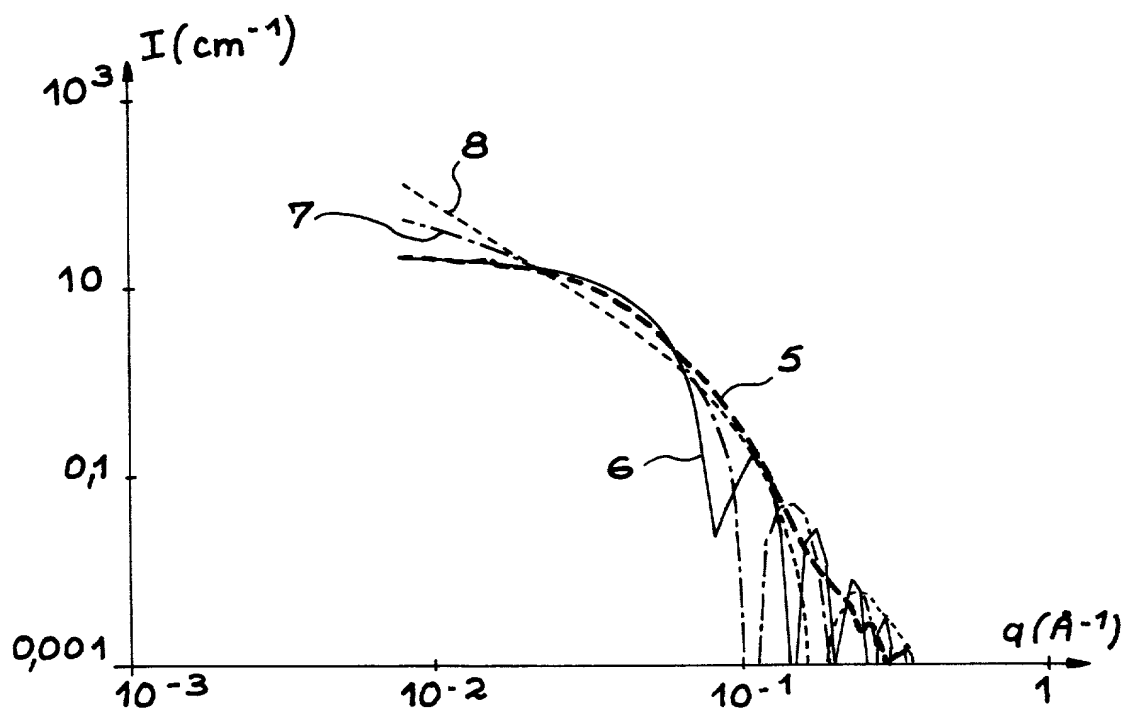


FIG. 6

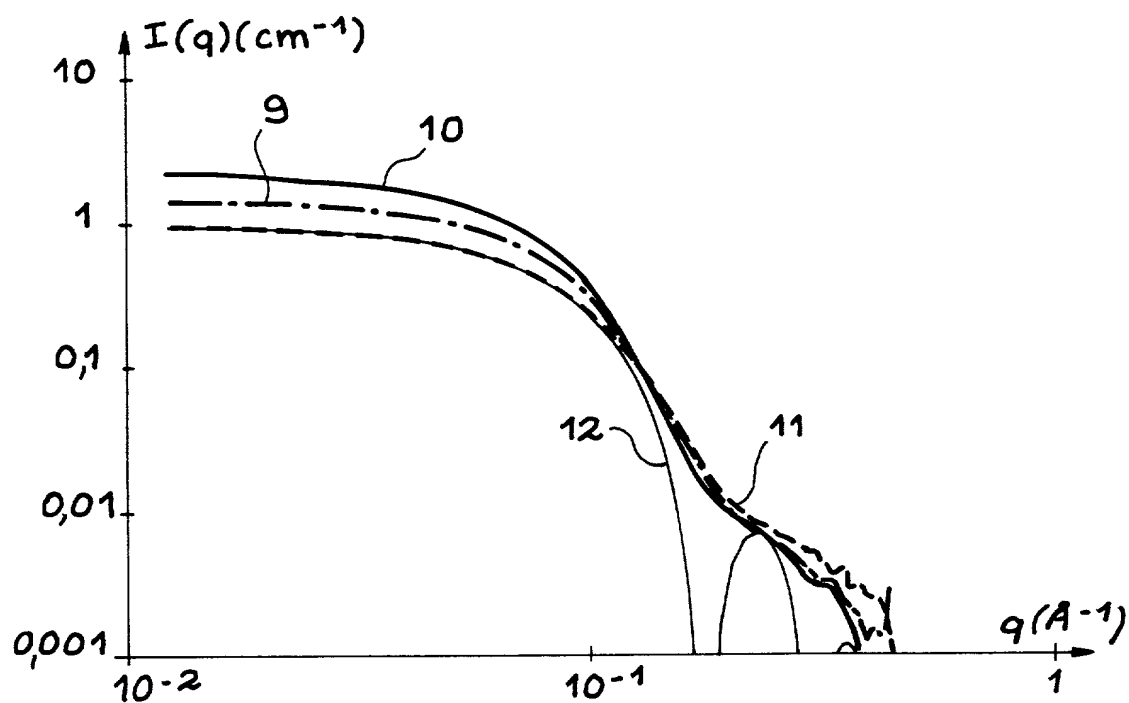


FIG. 7

Declaration, Power Of Attorney and Petition

Page 1 of 3

WE (I) the undersigned inventor(s), hereby declare(s) that :

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

AMPHIPHILIC CYCLODEXTRINS, PREPARATION AND USE OF SAID CYCLODEXTRINS TO SOLUBILISE ORGANISED SYSTEMS AND INCORPORATE HYDROPHOBIC MOLECULES

the specification of which

- ☐ is attached hereto.
- ☐ was filed on
as Application Serial No.
and amended on
- ☒ was filed as PCT international application
Number PCT/FR00/01102
on April 26, 2000
and was amended under PCT Article 19
on March 07, 2001

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365 (a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application (s)

Application No.	Country	Day/month/Year	Priority Claimed	
99 05460	FRANCE	29 APRIL 1999	<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
_____	_____	_____	<input type="checkbox"/> YES	<input type="checkbox"/> NO
_____	_____	_____	<input type="checkbox"/> YES	<input type="checkbox"/> NO
_____	_____	_____	<input type="checkbox"/> YES	<input type="checkbox"/> NO

We (I) hereby claim the benefit under Title 35, United States Code, § 119 (e) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of prior application and the national or PCT International filing date of this application.

Application Serial No.

Filing Date

Status (pending, patented,
abandoned)

And we (I) hereby appoint : Norman F. Oblon, Registration Number 24,618; Marvin J. Spivak, Registration Number 24,913; C. Irvin McClelland, Registration Number 21,214; Gregory J. Maier, Registration Number 25,599; Arthur I. Neustadt, Registration Number 24,854; Richard D. Kelly, Registration Number 27,757; James D. Hamilton, Registration Number 28,421; Eckhard H. Kuesters, Registration Number 28,870; Robert T. Pous, Registration Number 29,099; Charles L. Gholz, Registration Number 26,395; Vincent J. Sunderdick, Registration Number 29,004; William E. Beaumont, Registration Number 30,996; Steven B. Kelber, Registration Number 30,073; Robert F. Gnuse, Registration Number 27,295; Jean-Paul Lavalleye, Registration Number 31,451; William B. Walker, Registration Number 22,498; Timothy R. Schwartz, Registration Number 32,171; Stephen G. Baxter, Registration Number 32,884; Martin M., Zoltick, Registration Number 35,745; Robert W. Hahl, Registration Number 33,893; and Richard L. Treanor, Registration Number 36,379; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, MCCLELLAND, MAIER & NEUSTADT, P.C., whose post Office Address is : Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

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